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# Archives OF INTERNAL MEDICINE

JANUARY 1956

VOLUME 97 NUMBER 1

**Free Reducing and Hydrocortisone-like Steroids in Human Plasma**

*Smith Freeman, J. X. Wheeler, and H. W. Hoegemeier*

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**A Bibliography of Internal Medicine**

*Arthur L. Bloomfield*

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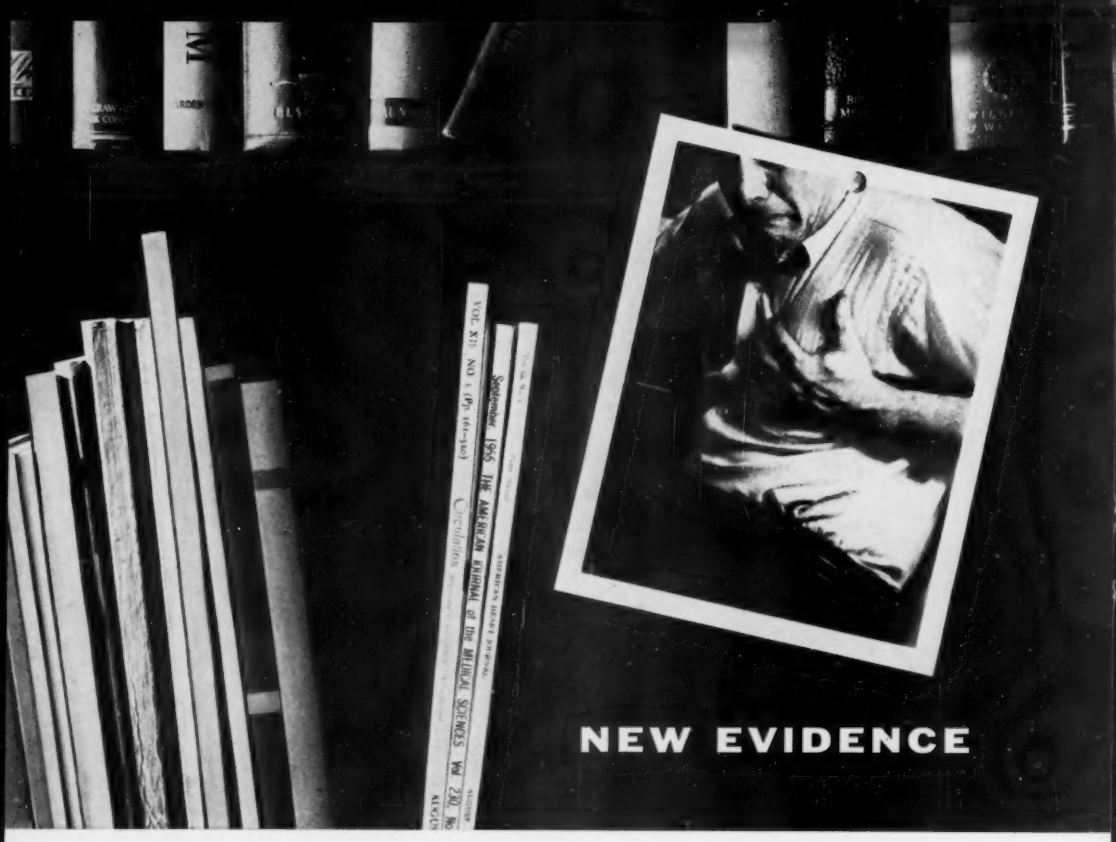


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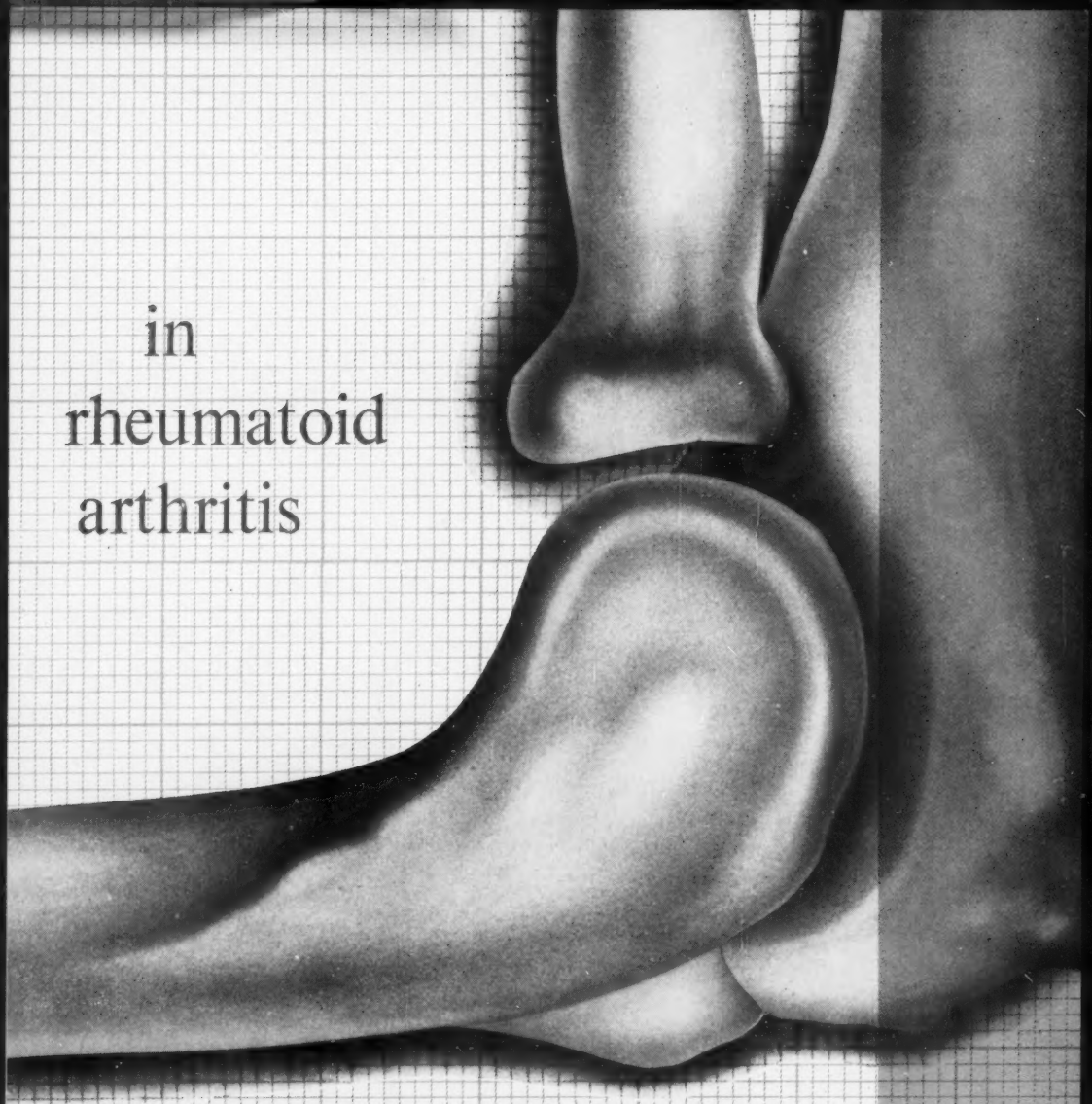

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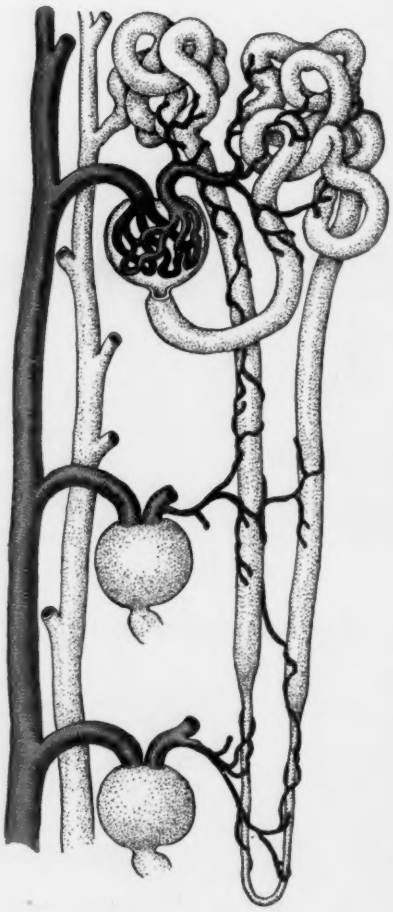

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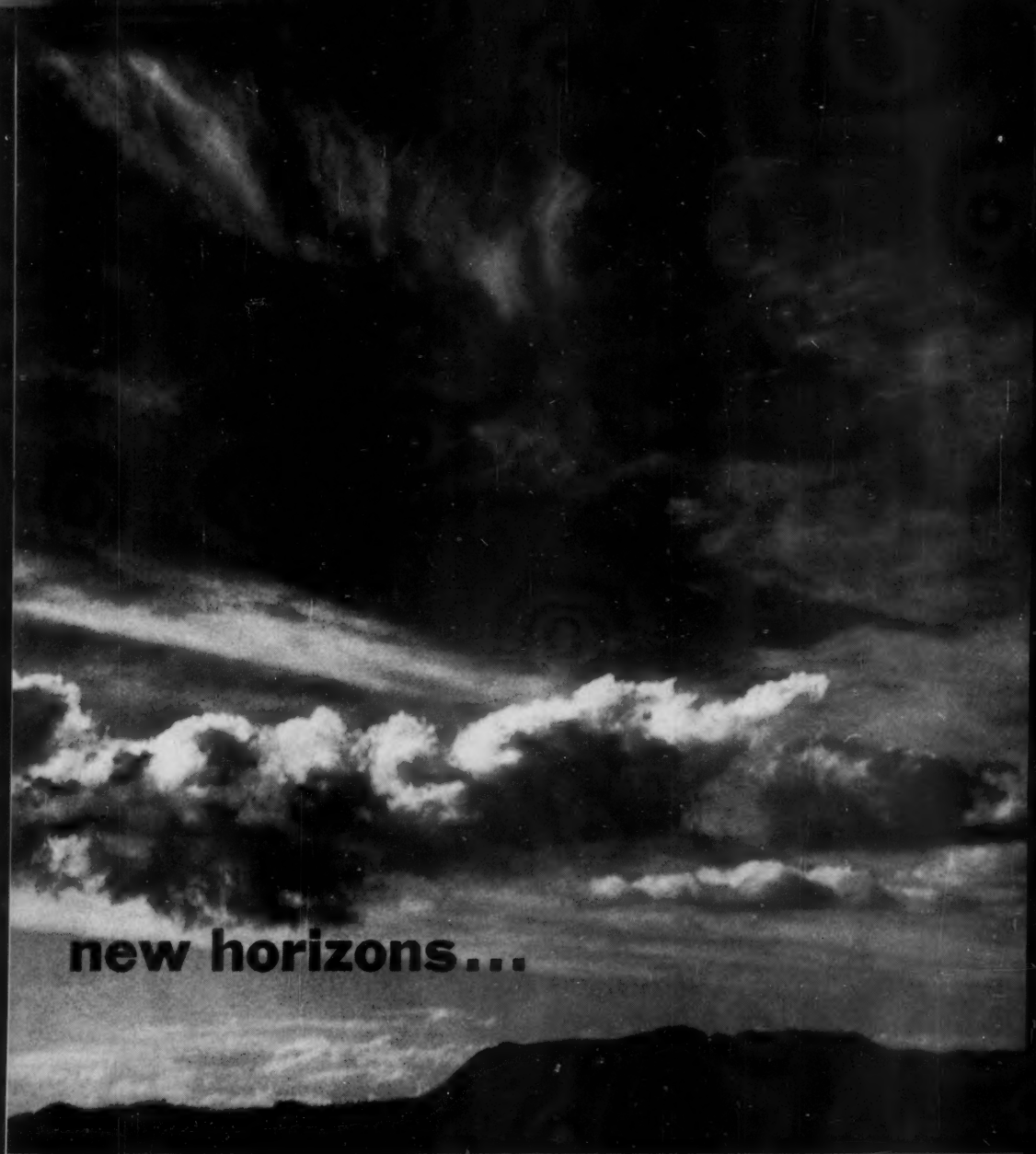


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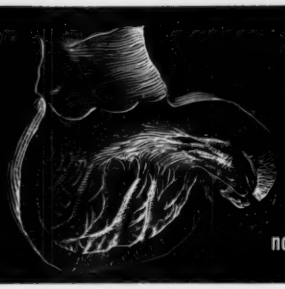

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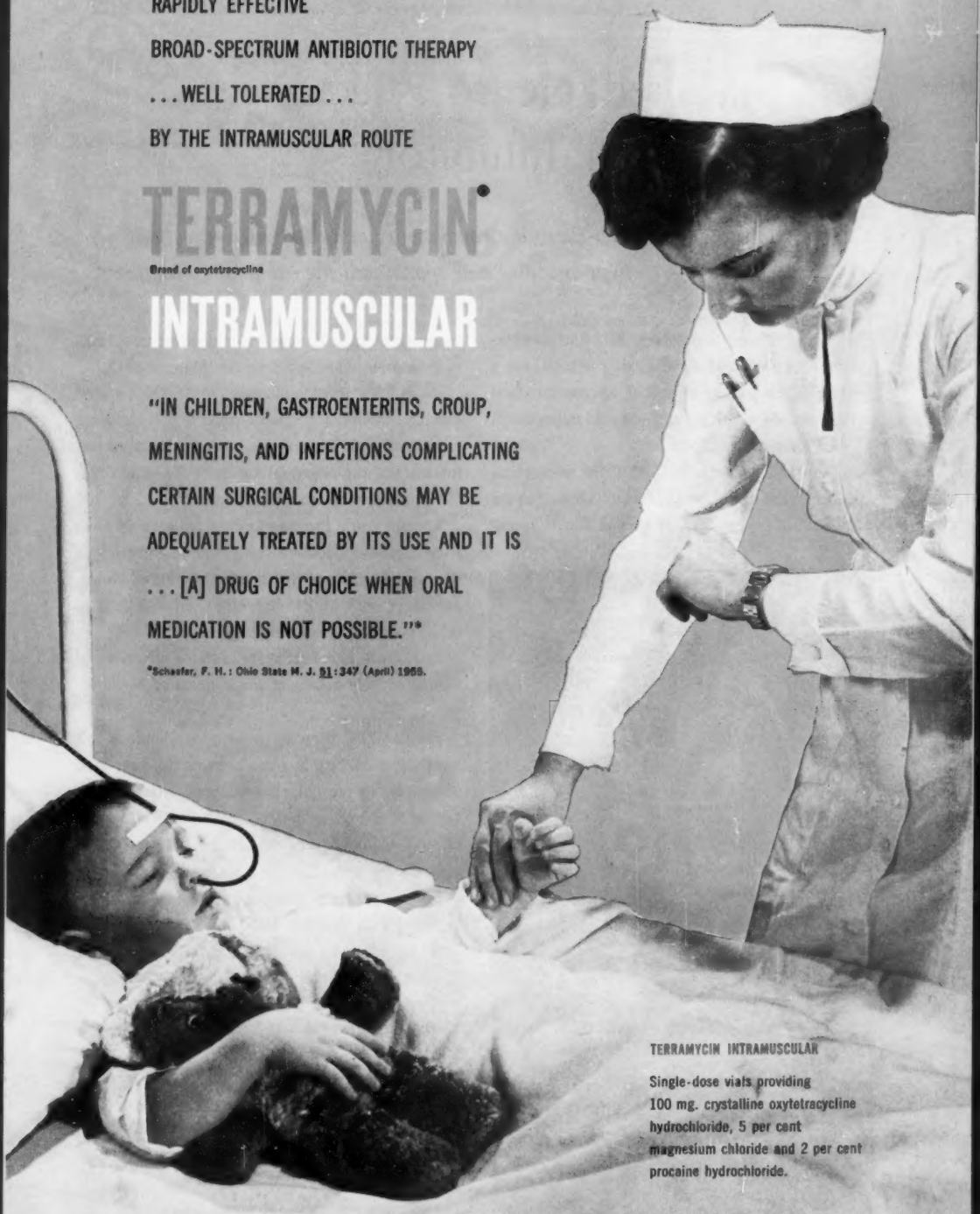

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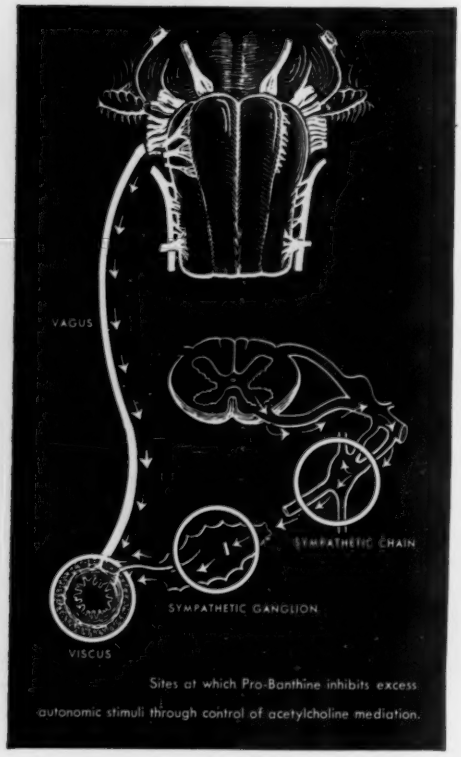


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1. Schwartz, I. R.; Lehman, E.; Ostrove, R., and Seibel, J. M.: Gastroenterology *25*:416 (Nov.) 1953.

2. Roback, R. A., and Beal, J. M.: Gastroenterology *25*:24 (Sept.) 1953.

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A.M.A. Archives OF INTERNAL MEDICINE

# Studies on the Destruction of Red Blood Cells

## IX. Quantitative Methods for Determining the Osmotic and Mechanical Fragility of Red Cells in the Peripheral Blood and Splenic Pulp; The Mechanism of Increased Hemolysis in Hereditary Spherocytosis (Congenital Hemolytic Jaundice) as Related to the Functions of the Spleen

CHARLES P. EMERSON Jr., M.D., Boston
SHU CHU SHEN, M.D., Cambridge, Mass.
THOMAS HALE HAM, M.D., Cleveland
ELEANOR M. FLEMING, B.A.
and
WILLIAM B. CASTLE, M.D., Boston

Hereditary spherocytosis (congenital hemolytic jaundice) is an inherited disease entity characterized by chronic anemia, by increased hemolysis and erythropoiesis, and frequently by splenomegaly. The classic feature of this disease is the presence in the peripheral blood of red cells that are spheroidal in shape [1] and that are unusually susceptible to hemolysis when suspended in hypotonic media.[2] These two abnormal properties have been shown to be mutually interdependent, the increased osmotic fragility reflecting the fact that the surface membrane of the erythrocyte is reduced in size relative to its volume,* with the result that its capacity to swell before reaching the rupture point is reduced. Also, these cells are abnormally susceptible to trauma, having an increased "mechanical fragility." Moreover, when blood from these patients is incubated at 37.5 C there occurs a pronounced increase in the osmotic and mechanical fragility of the red cells which surpasses to a significant degree the changes occurring in normal blood.

The pathologic significance of the erythrocytic defect in hereditary spherocytosis has not been clarified. Red cells with similar characeristics are observed in a variety of hemolytic anemias which bear no etiologic interrelationship. For example, spheroidicity and an increase in the osmotic fragility of red cells have been described as a result of thermal injury,† intoxication by various drugs and chemical oxidants,‡ transfusions of plasma containing incompatible isoagglutinins,§ experimental injections of anti-red-cell immune serum,[12] and in certain cases of acquired hemolytic jaundice of the so-called "autoimmune" variety, in which antibody globulin is demonstrable by agglu-

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From the Thorndike Memorial Laboratory and the Second and Fourth (Harvard) Medical Services, Boston City Hospital, and the Department of Medicine, Harvard Medical School; the Robert Dawson Evans Memorial Laboratory, Massachusetts Memorial Hospitals, and Boston University School of Medicine; Holy Ghost Hospital, Cambridge, Mass., and Tufts Medical and Dental School, Boston, and Western Reserve University School of Medicine and the University Hospitals, Cleveland.

* References 3 and 4.
† References 5 and 6.
‡ References 7 and 10.
§ References 11 and 12.

tination of these cells by globulin antiserum, i. e., a positive Coombs test.‖

However, neither toxic processes nor immune mechanisms have been established in typical cases of hereditary spherocytosis. The Coombs test, which was applied in the majority of cases described in this report, was negative. Tests for hemolysis with acidified serum and complement ¶ likewise proved negative, excluding, in these cases, the presence of circulating hemolysins of the type described in some patients with acquired hemolytic jaundice,[15] as well as the red cell abnormality characteristic of paroxysmal nocturnal hemoglobinuria.#

Thus it has not been possible to explain the observed alterations in red cell morphology and osmotic fragility on the basis of any humoral mechanism. Moreover, the likelihood that such a mechanism could be responsible for excessive blood destruction in this disease seems remote in the light of the observations reported by Dacie and Mollison [20] and by others * indicating that normal donor red cells when transfused into these patients survive normally. Conversely, when red cells from a case of hereditary spherocytosis are injected into a normal person they are destroyed with unusual rapidity, another manifestation of their defective quality.†

Removal of the spleen in cases of hereditary spherocytosis is almost invariably followed by rapid alleviation or elimination of the anemia and jaundice. There is a simultaneous decrease in the excretion of bile pigment and in the number of circulating reticulocytes, indicating that the effect of this procedure is to decrease blood destruction rather than to increase blood production. Regardless of their improved status, however, these patients retain their cell defect following splenectomy, as manifested by persistant spheroidicity and by abnormal increases in osmotic and mechanical fragility before and after incubation. Morever, as shown by Dacie and Mollison,[20] red cells transfused from these patients into normal recipients are destroyed with equal rapidity whether they were obtained from the patient before or after splenectomy.

With respect to the mechanism by which the spleen contributes to blood destruction in hereditary spherocytosis, Wagley and co-workers,[24] employing the indirect Coombs test, failed to detect any trace of red cell antibody in the splenic pulp in these cases, in contrast to cases of acquired hemolytic anemia. Young and co-workers [25] reported that when mixtures of normal red cells and red cells from patients with hereditary spherocytosis were perfused through spleens which were freshly removed from patients with thrombocytopenic purpura the abnormal ones were selectively extracted from the perfusate. These observations suggest that the effect of the spleen is not a manifestation of splenic dysfunction but is dependent upon some abnormal property of the erythrocyte. It is possible that red cell morphology may be the determining factor in this process; thus, spheroidicity, in contrast to normal biconcavity, may predispose to sequestration and destruction of erythrocytes in the spleen.

On the basis of evidence now available it is believed that the essential abnormality in patients with hereditary spherocytosis is an inherited defect of the red cell which causes the later to be retained in, and conditioned by, the spleen. The precise characteristic that is responsible for its sequestration from the circulating blood may be its morphologic configuration, namely, its spheroidicity. In the studies reported here, observations have been made in vivo to determine whether or not cells with a pronounced abnormality are to be found in the spleen, and, if so, whether this results from normal or abnormal activity on the part of the spleen. Comparisons were also made between blood from the splenic vein and blood obtained from the splenic pulp, in order to elucidate further the mechanisms

‖ References 13 and 14.

¶ References 15-17.

# References 18 and 19.

* References 21-23.

† References 20-23.

of hemolysis in hereditary spherocytosis as related to the functions of the spleen.

### CASES AND STANDARD METHODS

Thirty-one patients with hereditary spherocytosis were studied. Thirteen of these cases were observed before, during, and after splenectomy; three were examined only after splenectomy that had been performed from 4 to 20 years earlier. In the remaining 15 the spleens had not been removed.

Red cells in blood from the splenic vein and red cells extracted from the pulp of the spleen were studied at the time of operation in nine cases. Five of these patients had received preoperative transfusions of normal compatible red cells selected on the basis of difference in blood groups which permitted their selective agglutination and quantitative estimation by the Ashby method. Tests of compatibility were performed, including the Coombs test and cross matching of cells in serum.

Control data were obtained from studies of the peripheral blood of 31 normal subjects. Control observations were also made on blood samples from the spleen in eight cases without hemolytic anemia, including six patients with idiopathic thrombocytopenic purpura and two with pancytopenia of unknown origin.

*Standard Methods.*—Peripheral blood was obtained from the antecubital vein, one sample being defibrinated with glass beads and another sample of 5 ml. being collected in a 6 ml. bottle containing a mixture of dry ammonium and potassium oxalate, 200 mg. per 100 ml. of blood.[16] Determinations of the red cell count, hematocrit value, and hemoglobin concentration were performed on venous blood containing the dry oxalate mixture. The concentrations of hemoglobin and of plasma or serum bilirubin were measured by photometric methods,‡ with use of an Evelyn photoelectric cell colorimeter.[26] Other procedures[16] included the Donath-Landsteiner test, test for hemolysis in acidified serum, test for sickling, tests for autohemagglutinins and autohemolysins, and the Coombs test. Sterile defibrinated blood was used for the incubation of red cells and as a source of serum for immune studies.

‡ References 26 and 27.

### QUANTITATIVE METHODS FOR DETERMINING OSMOTIC AND MECHANICAL FRAGILITIES OF RED CELLS IN PERIPHERAL BLOOD AND SPLENIC PULP

#### Osmotic Fragility

The osmotic fragility test was performed by suspending red cells in hypotonic solutions of sodium chloride and determining the degree of hemolysis in each mixture by quantitative measurement of the extracellular hemoglobin. The tonicity of the blood-saline mixtures was corrected for the addition of blood and for variations of pH. The osmotic fragility was determined on samples of defibrinated or oxalated blood obtained from the antecubital vein, splenic vein, splenic pedicle, and parenchyma of the spleen. The pH was measured immediately prior to the test and was used in correcting the results, as described below. Occasionally the pH was adjusted as described in the paragraph on correction of osmotic fragility.

In the standard procedure, 0.1 ml. samples of blood were measured accurately between marks in a Mohr pipette, added to 1.0 ml. samples of aqueous solutions of sodium chloride, and promptly mixed. The salt solutions ranged in concentration from hypertonic (1.0 gm/100 ml.) to zero (distilled water), differing by increments of 0.04 or 0.05 gm. of sodium chloride per 100 ml. These solutions contained the following concentrations of sodium chloride, expressed as gm/100 ml.: 1.0, 0.95, 0.85, 0.80, 0.75, 0.70, 0.66, 0.62, 0.58, 0.54, 0.50, 0.46, 0.42, 0.38, 0.34, 0.30, 0.26, 0.18, and 0.00 (distilled water).

The mixtures of blood and saline were centrifuged soon after they were prepared. The supernatant solutions were then decanted and their hemoglobin content determined, after appropriate dilution, in an Evelyn or Coleman (Model No. 6) photoelectric colorimeter. The optical density of the supernatant solution from the hypertonic saline suspension was employed as a blank and was subtracted from the values obtained in the other tests, the difference in each case representing hemoglobin which had been released by hemolysis. The degree of hemolysis in

TABLE 1.—*Steps Taken in the Measurement and Analysis of Osmotic Fragility* *

| Data on Osmotic Fragility Used for Preparation of Summation Curve | | | Data Extrapolated from Summation Curve, Used in Tabulation | | Data Extrapolated from Summation Curve Used to Plot the the Curve of Distribution Frequency | | |
|---|---|---|---|---|---|---|---|
| | | | | | Increment of Corrected Tonicity | Hemolysis, per Cent | |
| Hemolysis, per Cent (1) | Uncorrected Tonicity (Tu),† pH 7.3 (2) | Corrected Tonicity (Tc),† pH 7.4 (3) | Hemolysis, per Cent (4) | Corrected Tonicity (Tc)† (5) | (Tc)† (6) | Total (7) | Difference (8) |
| 0 | 1.00 | 0.960 | 1 | 0.84 | 0.88 | 0.2 | 0.2 |
| 1.5 | 0.85 | 0.830 | 2 | 0.82 | 0.86 | 0.7 | 0.5 |
| 3.5 | 0.80 | 0.785 | 5 | 0.77 | 0.84 | 1.2 | 0.5 |
| 7.5 | 0.75 | 0.740 | 10 | 0.73 | 0.82 | 2.0 | 0.8 |
| 20 | 0.70 | 0.695 | 25 | 0.68 | 0.80 | 2.7 | 0.7 |
| 37 | 0.66 | 0.660 | 50 | 0.64 | 0.78 | 3.2 | 0.5 |
| 53 | 0.62 | 0.635 | 75 | 0.52 | 0.76 | 5.5 | 2.3 |
| 66 | 0.58 | 0.590 | 90 | 0.49 | 0.74 | 8.0 | 2.5 |
| 69 | 0.54 | 0.565 | | | 0.72 | 12.0 | 4.0 |
| 76 | 0.50 | 0.520 | | | 0.70 | 18.0 | 6.0 |
| 91 | 0.46 | 0.485 | | | 0.68 | 26.0 | 8.0 |
| 100 | 0.42 | 0.450 | | | 0.66 | 37.5 | 11.5 |
| | | | | | 0.64 | 50.5 | 13.0 |
| | | | | | 0.62 | 58.5 | 8.0 |
| | | | | | 0.60 | 64.0 | 5.5 |
| | | | | | 0.58 | 67.0 | 3.0 |
| | | | | | 0.56 | 68.5 | 1.5 |
| | | | | | 0.54 | 72.0 | 3.5 |
| | | | | | 0.52 | 76.5 | 4.5 |
| | | | | | 0.50 | 84.0 | 7.5 |
| | | | | | 0.48 | 92.5 | 8.5 |
| | | | | | 0.46 | 97.5 | 5.0 |
| | | | | | 0.44 | 100.0 | 2.5 |

* Data were obtained in Case 9 on the blood sample obtained from the splenic pulp (see Table 4 and Fig. 5). Hemolysis (1) was determined colorimetrically in stock solutions of sodium chloride (2) and the tonicity was corrected (3) from pH 7.3 to 7.4. A summation curve of the percentage hemolysis was drawn from the data in columns (1) and (3) as shown in Figure 5. From this curve the data in columns (4) and (5) were extrapolated and recorded in Table 4 (Case 9). Also from the summation curve the hemolysis was extrapolated in column (7) for increments of tonicity equivalent to concentrations of sodium chloride of 0.02 gm./100 ml. in column (6). The differences in hemolysis in column (8) for the increments of tonicity were plotted as a curve of distribution, which appears in the right-hand panel of Figure 5.

† Tonicity expressed as sodium chloride, gm./100 ml.

each mixture was calculated, in per cent, by dividing the observed concentration of hemoglobin by the total available hemoglobin as measured in the sample diluted with distilled water. Examples of these data are shown in columns (1) and (2) of Table 1.

*Correction of the Osmotic Fragility for Tonicity and pH.*—The uncorrected tonicity (Tu) of each mixture of saline and blood was corrected (Tc) for the added blood and to a pH of 7.4, with use of the following formula for a dilution of blood of 1:11. Examples of these data are shown in column (3) of Table 1.

$$\text{Tonicity at pH 7.4 (or } Tc) = \frac{(Tu \times 10) + 0.85}{11.0} \times \frac{11.5 - 7.4}{11.5 - \text{pH}} \text{ or } = (10\ Tu + 0.85) \times \frac{0.373}{11.5 - \text{pH}}$$

where

*Tc*=the corrected tonicity for the blood-saline mixture, expressed as NaCl, gm/100 ml.

*Tu*=the concentration of sodium chloride in the stock saline solution, and

pH=pH of the blood sample just before it is tested.

The first expression in the formula is based on the observation that the red cells and plasma or serum are equivalent approximately to a solution of sodium chloride of 0.85 gm/100 ml. The second expression § in the formula is based on the observations of Jacobs and Parpart [28] and Emerson ‖ indicating that there is a linear relation between the volume of red cells and the pH. As the pH decreases the red cells swell and their osmotic fragility is increased. A table has been prepared (Table 2) from the above formula for correction of the tonicity to the calculated tonicity for pH 7.4. It has been

§ The detailed data (Emerson, C. P.: Unpublished data) and derivation of this expression will be published elsewhere. The formula has been verified for the range of pH, as shown in Table 2.

‖ Emerson, C. P.: Unpublished data.

TABLE 2.—*Table for Obtaining the Corrected Tonicity (Tc) at pH 7.4 for Red Cells Suspended in Saline* *

| Tu (NaCl, Gm./ 100 Ml.) | Corrected Tonicity (Tc), Gm. NaCl/100 Ml. | | | | | | | | | | | | | | | Tu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH 6.6 | pH 6.8 | pH 7.0 | pH 7.1 | pH 7.2 | pH 7.3 | pH 7.4 | pH 7.5 | pH 7.6 | pH 7.7 | pH 7.8 | pH 7.9 | pH8.0 | pH 8.1 | pH 8.2 | |
| 1.00 | 0.825 | 0.860 | 0.900 | 0.920 | 0.940 | 0.965 | 0.990 | 1.010 | 1.040 | 1.065 | 1.095 | 1.125 | 1.155 | 1.190 | 1.225 | 1.00 |
| 0.90 | 0.750 | 0.780 | 0.815 | 0.835 | 0.855 | 0.875 | 0.895 | 0.920 | 0.940 | 0.965 | 0.990 | 1.020 | 1.050 | 1.080 | 1.115 | 0.90 |
| 0.88 | 0.735 | 0.765 | 0.800 | 0.820 | 0.840 | 0.855 | 0.880 | 0.900 | 0.925 | 0.945 | 0.970 | 1.000 | 1.025 | 1.055 | 1.090 | 0.88 |
| 0.86 | 0.720 | 0.750 | 0.785 | 0.800 | 0.820 | 0.840 | 0.860 | 0.880 | 0.905 | 0.925 | 0.955 | 0.980 | 1.005 | 1.035 | 1.065 | 0.86 |
| 0.84 | 0.700 | 0.735 | 0.765 | 0.785 | 0.805 | 0.820 | 0.840 | 0.860 | 0.885 | 0.905 | 0.930 | 0.960 | 0.985 | 1.015 | 1.045 | 0.84 |
| 0.82 | 0.690 | 0.720 | 0.750 | 0.765 | 0.785 | 0.805 | 0.825 | 0.845 | 0.865 | 0.890 | 0.910 | 0.935 | 0.965 | 0.990 | 1.020 | 0.82 |
| 0.80 | 0.670 | 0.700 | 0.735 | 0.750 | 0.770 | 0.785 | 0.805 | 0.825 | 0.845 | 0.870 | 0.890 | 0.915 | 0.940 | 0.970 | 1.000 | 0.80 |
| 0.78 | 0.655 | 0.685 | 0.715 | 0.735 | 0.750 | 0.770 | 0.785 | 0.805 | 0.830 | 0.850 | 0.870 | 0.895 | 0.920 | 0.950 | 0.980 | 0.78 |
| 0.76 | 0.640 | 0.670 | 0.700 | 0.715 | 0.735 | 0.750 | 0.770 | 0.785 | 0.810 | 0.830 | 0.850 | 0.875 | 0.900 | 0.925 | 0.955 | 0.76 |
| 0.74 | 0.625 | 0.655 | 0.685 | 0.700 | 0.715 | 0.735 | 0.750 | 0.770 | 0.790 | 0.810 | 0.830 | 0.855 | 0.880 | 0.905 | 0.930 | 0.74 |
| 0.72 | 0.610 | 0.640 | 0.665 | 0.685 | 0.700 | 0.715 | 0.730 | 0.750 | 0.770 | 0.790 | 0.810 | 0.835 | 0.855 | 0.880 | 0.910 | 0.72 |
| 0.70 | 0.595 | 0.625 | 0.650 | 0.665 | 0.680 | 0.695 | 0.715 | 0.730 | 0.750 | 0.770 | 0.790 | 0.810 | 0.835 | 0.860 | 0.885 | 0.70 |
| 0.68 | 0.580 | 0.605 | 0.635 | 0.650 | 0.665 | 0.680 | 0.695 | 0.715 | 0.730 | 0.750 | 0.770 | 0.790 | 0.815 | 0.840 | 0.865 | 0.68 |
| 0.66 | 0.565 | 0.590 | 0.620 | 0.630 | 0.645 | 0.660 | 0.680 | 0.695 | 0.715 | 0.730 | 0.750 | 0.770 | 0.795 | 0.815 | 0.840 | 0.66 |
| 0.64 | 0.550 | 0.575 | 0.600 | 0.615 | 0.630 | 0.645 | 0.660 | 0.675 | 0.695 | 0.710 | 0.730 | 0.750 | 0.770 | 0.795 | 0.820 | 0.64 |
| 0.62 | 0.535 | 0.560 | 0.585 | 0.600 | 0.615 | 0.625 | 0.640 | 0.660 | 0.675 | 0.690 | 0.710 | 0.730 | 0.750 | 0.770 | 0.795 | 0.62 |
| 0.60 | 0.520 | 0.545 | 0.570 | 0.580 | 0.595 | 0.610 | 0.625 | 0.640 | 0.655 | 0.670 | 0.690 | 0.710 | 0.730 | 0.750 | 0.775 | 0.60 |
| 0.58 | 0.505 | 0.530 | 0.550 | 0.565 | 0.575 | 0.590 | 0.605 | 0.620 | 0.635 | 0.650 | 0.670 | 0.690 | 0.710 | 0.730 | 0.750 | 0.58 |
| 0.56 | 0.490 | 0.510 | 0.535 | 0.550 | 0.560 | 0.570 | 0.590 | 0.600 | 0.615 | 0.635 | 0.650 | 0.670 | 0.685 | 0.705 | 0.725 | 0.56 |
| 0.54 | 0.475 | 0.495 | 0.520 | 0.530 | 0.540 | 0.555 | 0.560 | 0.585 | 0.600 | 0.615 | 0.630 | 0.650 | 0.665 | 0.685 | 0.705 | 0.54 |
| 0.52 | 0.460 | 0.480 | 0.500 | 0.515 | 0.525 | 0.535 | 0.550 | 0.565 | 0.580 | 0.595 | 0.610 | 0.625 | 0.645 | 0.665 | 0.685 | 0.52 |
| 0.50 | 0.445 | 0.465 | 0.485 | 0.495 | 0.510 | 0.520 | 0.530 | 0.545 | 0.560 | 0.575 | 0.590 | 0.605 | 0.625 | 0.640 | 0.660 | 0.50 |
| 0.48 | 0.430 | 0.450 | 0.470 | 0.480 | 0.490 | 0.500 | 0.515 | 0.530 | 0.540 | 0.555 | 0.570 | 0.585 | 0.600 | 0.620 | 0.640 | 0.48 |
| 0.46 | 0.415 | 0.430 | 0.450 | 0.460 | 0.475 | 0.485 | 0.495 | 0.510 | 0.520 | 0.535 | 0.550 | 0.565 | 0.580 | 0.595 | 0.615 | 0.46 |
| 0.44 | 0.400 | 0.415 | 0.435 | 0.445 | 0.455 | 0.465 | 0.480 | 0.490 | 0.500 | 0.515 | 0.530 | 0.545 | 0.560 | 0.575 | 0.595 | 0.44 |
| 0.42 | 0.385 | 0.400 | 0.420 | 0.430 | 0.440 | 0.450 | 0.460 | 0.470 | 0.485 | 0.495 | 0.510 | 0.520 | 0.540 | 0.555 | 0.570 | 0.42 |
| 0.40 | 0.370 | 0.385 | 0.400 | 0.410 | 0.420 | 0.430 | 0.440 | 0.450 | 0.465 | 0.475 | 0.490 | 0.500 | 0.515 | 0.530 | 0.550 | 0.40 |
| 0.38 | 0.355 | 0.370 | 0.385 | 0.395 | 0.405 | 0.415 | 0.425 | 0.435 | 0.445 | 0.455 | 0.470 | 0.480 | 0.495 | 0.510 | 0.525 | 0.38 |
| 0.36 | 0.340 | 0.355 | 0.370 | 0.380 | 0.385 | 0.395 | 0.405 | 0.415 | 0.425 | 0.435 | 0.450 | 0.460 | 0.475 | 0.490 | 0.500 | 0.36 |
| 0.34 | 0.325 | 0.350 | 0.355 | 0.360 | 0.370 | 0.375 | 0.385 | 0.395 | 0.405 | 0.415 | 0.430 | 0.440 | 0.455 | 0.465 | 0.480 | 0.34 |
| 0.32 | 0.310 | 0.320 | 0.335 | 0.345 | 0.350 | 0.360 | 0.370 | 0.380 | 0.385 | 0.400 | 0.410 | 0.420 | 0.430 | 0.445 | 0.460 | 0.32 |
| 0.30 | 0.295 | 0.305 | 0.320 | 0.325 | 0.335 | 0.340 | 0.350 | 0.360 | 0.370 | 0.380 | 0.390 | 0.400 | 0.410 | 0.420 | 0.435 | 0.30 |
| 0.28 | 0.275 | 0.290 | 0.305 | 0.310 | 0.315 | 0.325 | 0.330 | 0.340 | 0.350 | 0.360 | 0.370 | 0.380 | 0.390 | 0.400 | 0.415 | 0.28 |
| 0.26 | 0.260 | 0.275 | 0.285 | 0.295 | 0.300 | 0.305 | 0.315 | 0.320 | 0.330 | 0.340 | 0.350 | 0.355 | 0.370 | 0.380 | 0.390 | 0.26 |
| 0.24 | 0.245 | 0.260 | 0.270 | 0.275 | 0.280 | 0.290 | 0.295 | 0.305 | 0.310 | 0.320 | 0.330 | 0.335 | 0.345 | 0.355 | 0.365 | 0.24 |
| 0.22 | 0.230 | 0.240 | 0.255 | 0.260 | 0.265 | 0.270 | 0.280 | 0.285 | 0.290 | 0.300 | 0.310 | 0.315 | 0.325 | 0.335 | 0.345 | 0.22 |
| 0.20 | 0.215 | 0.225 | 0.235 | 0.240 | 0.245 | 0.255 | 0.260 | 0.265 | 0.275 | 0.280 | 0.285 | 0.295 | 0.305 | 0.315 | 0.320 | 0.20 |
| 0.18 | 0.200 | 0.210 | 0.220 | 0.225 | 0.230 | 0.240 | 0.240 | 0.245 | 0.255 | 0.260 | 0.265 | 0.275 | 0.280 | 0.290 | 0.300 | 0.18 |
| 0.15 | 0.180 | 0.185 | 0.195 | 0.200 | 0.205 | 0.210 | 0.215 | 0.220 | 0.225 | 0.230 | 0.235 | 0.245 | 0.250 | 0.260 | 0.265 | 0.15 |
| 0.10 | 0.140 | 0.145 | 0.155 | 0.155 | 0.160 | 0.165 | 0.170 | 0.170 | 0.175 | 0.180 | 0.185 | 0.190 | 0.195 | 0.205 | 0.210 | 0.10 |
| 0.05 | 0.105 | 0.105 | 0.110 | 0.115 | 0.115 | 0.120 | 0.125 | 0.125 | 0.130 | 0.130 | 0.135 | 0.140 | 0.145 | 0.150 | 0.155 | 0.05 |
| pH | 6.6 | 6.8 | 7.0 | 7.7 | 7.2 | 7.3 | 7.4 | 7.5 | 7.6 | 7.7 | 7.8 | 7.9 | 8.0 | 8.1 | 8.2 | pH |

* The data are derived from the concentration of sodium chloride (Tu) and the observed pH of the blood sample. Table is based on the formula in text.
Example: Tc (at pH 7.4) is obtained by substituting for Tu the figures listed in the column corresponding to the pH of the blood sample. Thus, if 0.9% hemolysis occurs when blood at pH 7.8 is diluted with 10 volumes of salt solution containing 0.68 gm. NaCl/100 ml. (Tu), Tc at 0.9% hemolysis for that sample is equivalent to 0.77 gm. NaCl/100 ml.

observed ¶ that acidification of defibrinated blood by equilibration with carbon dioxide or lactic acid, reducing the pH from the range of approximately 7.4 to 8.0 down to a range of 7.0 to 6.2 units, produced a predictable increase in the osmotic fragility and an increase in red cell volume but no hemolysis. Adjustment of the pH to approximately 7.4 by equilibration of the sample with room air, or by washing and resuspension of the red cells in fresh serum, restored the osmotic fragility to its original, or to a predictable, level. In general, the pH of the red cell suspension was adjusted within a range of 7.1 to 7.4 by equilibration of the sample with room air or an appropriate mixture of carbon dioxide and oxygen. Since no precautions were taken against the loss of carbon dioxide in the pipetting of blood samples, a rise of approximately 0.2 units occurred in the range of pH, 7.3 to 7.5, during the whole procedure of setting up the osmotic fragility test. This change was disregarded, and the data were corrected arbitrarily according to the pH measured on the blood sample immediately before it was pipetted. All data on osmotic fragility were corrected to a pH of 7.4 and for added blood, as indicated in the formula and in Table 2.

Although the control of pH was semiquantitative, it was demonstrated that whole blood had marked buffering power in the dilution of 1 part of blood in 10 parts of saline, and because of this the limits of pH change during the performance of the test were narrow. This semiquantitative method of pH control # proved to be a reproducible

¶ References 17 and 29 and Emerson, C. P.: Unpublished data.

# The pH can be stabilized further by using defibrinated blood, which will have lost much of its carbon dioxide, or by pipetting the blood under the surface of the hypotonic solutions, which decreases the loss of carbon dioxide.

TABLE 3.—*An Example of the Steps Taken in the Determination of the Differential Osmotic Fragility of a Mixed Population of Red Cells That Can Be Separated by Differential Agglutination* *

| Data on Osmotic Fragility of Mixed Population of Red Cells | | No. of Unhemolyzed Donor Cells in Each Dilution for Osmotic Fragility, Cells/Cu. Mm. | Hemolysis of Donor Cells, per Cent | Hemolysis of Patient's Cells, per Cent |
|---|---|---|---|---|
| Hemolysis, per Cent (1) | Corrected Tonicity (Tc)† at pH 7.4 (2) | (3) | (4) | (5) |
| 0 | 0.870 | 35,000 | 0 | 0 |
| 0.3 | 0.825 | 46,000 | 0 | 0.3 |
| 1.3 | 0.780 | 43,000 | 0 | 1.5 |
| 7.0 | 0.730 | 36,000 | 0 | 7.8 |
| 17.0 | 0.695 | 33,000 | 0 | 19.0 |
| 33.3 | 0.655 | Mean, 39,000 — 42,000 | 0 | 37.0 |
| 49.0 | 0.620 | 37,000 | 0 | 55.0 |
| 56.7 | 0.580 | 46,000 | 0 | 63.5 |
| 62.5 | 0.545 | 33,000 | 0 | 70.0 |
| 68.0 | 0.510 | 35,000 | 0 | 76.0 |
| 81.5 | 0.470 | 29,000 | 25.5 | 89.5 |
| 92.0 | 0.435 | 14,000 | 64.0 | 95.5 |
| 97.0 | 0.395 | 4,000 | 90.0 | 98.0 |
| 100.0 | 0.360 | 0 | 100.0 | 100.0 |

* In this instance normal Group O donor cells were transfused into a Group A patient (Case 6) and a sample was obtained from the splenic pulp at the time of operation (see also Fig. 12 and Table 9).

Hemolysis (1) was measured, and the corrected tonicity (2) was computed for differential agglutination counts. The suspension of unhemolyzed red cells in each tube (1) was treated with anti-A serum (see text), and the number of unagglutinated donor cells (Group O) was determined and reported in column (3) as the number per cu. mm. of unhemolyzed cells in the mixture for the osmotic fragility.

The hemolysis of the donor's cells (4) was calculated from the data in column (3) as follows:

$$\frac{39{,}000-(3)}{39{,}000}\times 100$$

The hemolysis of the patient's red cells (5) in the mixture was calculated from the data in columns (1) and (3), using the following formula:

$$\text{Per cent hemolysis} = 100\times(1)\div 100 - \frac{D\times(3)}{39{,}000}$$

where

$D$ = percentage of donor cells in the initial mixture, in this instance 10.5%.

The mean of 39,000 was used in the first 10 values.

† Tonicity expressed as sodium chloride, gm./100 ml.

and practical procedure that added to the value of the osmotic fragility test.

*Plotting and Reporting of Osmotic Fragility.*—The data were then plotted on rectilinear scales with per cent hemolysis on the ordinate and corrected tonicity (Tc) in NaCl, gm/100 ml., on the abscissa. This allowed inspection and evaluation of the shape of the summation curve of osmotic hemolysis, or the osmotic fragility of the red cells. From the summation curve, values were extrapolated, as shown in columns (4) and (5) of Table 1, to give the corrected tonicities at which specified degrees of hemolysis occurred such as those chosen arbitrarily for this report, namely, hemolysis

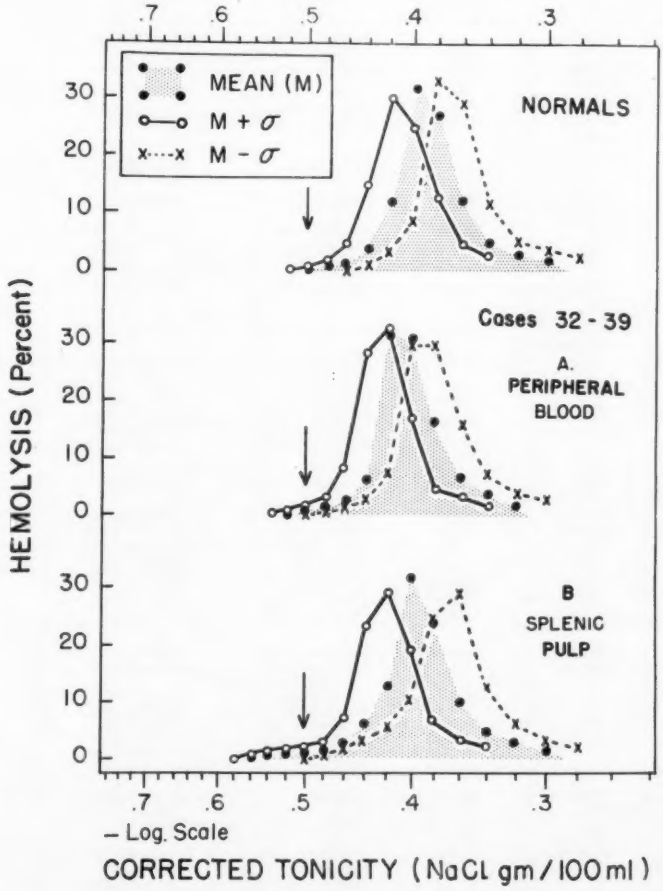


Fig. 1.—Osmotic fragility of red cells from control subjects. The red cells in the peripheral blood of 31 normal persons are compared with the cells in the peripheral and splenic pulp blood of 8 patients who did not have hemolytic disease (Cases 32 to 39, Table 5). These data serve as control observations for the methods and for patients with hemolytic anemia. Osmotic fragility is charted in the form of distribution curves showing, for each group, the percentage of red cells hemolyzed (mean value±1 S. D.) at corresponding tonicity levels by a reduction of tonicity equivalent to 0.02 gm. NaCl/100 ml. A vertical guide line, indicated by arrows, has been placed arbitrarily at the position corresponding to 0.50 gm. NaCl/100 ml., a tonicity level above which little or no hemolysis occurs normally. See Table 1 and text for the methods.

TABLE 4.—*Observations on Thirty-One Patients with Hereditary Spherocytosis, Representing*

| Case | Sex | Age, Yr. | Familial Incidence | Blood Sample Tested: Date | Source | Time Relation to Splenectomy | Serum Bilirubin, Total, Mg./100 Ml. | Icterus Index, Units |
|---|---|---|---|---|---|---|---|---|
| Group I. Patients observed before and after splenectomy | | | | | | | | |
| 1 | F | 54 | Mother of Cases 2 & 25; grandmother of Case 3 | 1/13/41 | Peripheral vein | Immediately before | 1.2 | 12 |
| | | | | | Peripheral vein | 1 hr. after | ... | .. |
| | | | | 2/ 8/41 | Peripheral vein | 8 days after | 0.3 | 4 |
| | | | | 2/14/41 | Peripheral vein | 14 days after | 0.3 | 4 |
| | | | | 10/25/41 | Peripheral vein | 9 mo. after | 0.4 | 5 |
| | | | | 10/14/46 | Peripheral vein | 5 yr. after | 0.3 | 5 |
| 2 | F | 32 | Daughter of Case 1; mother of Case 3 | 1/13/41 | Peripheral vein | 5 mo. before | 1.6 | 12 |
| | | | | 9/23/42 | Peripheral vein | 15 mo. after | 0.7 | 5 |
| | | | | 10/14/46 | Peripheral vein | 5 yr. after | 0.4 | 5 |
| 3 | F | 8 | Daughter of Case 2; granddaughter of Case 1 | 1/11/41 | Peripheral blood | 2½ yr. before | ... | 7 |
| | | | | 10/14/46 | Peripheral blood | 3 yr. after | 0.1 | 5 |
| | | | | 3/ 9/50 | Peripheral blood | 6 yr. after | ... | 8 |
| 4 | F | 5 | Daughter of Case 26 | 1/16/46 | Peripheral vein | 14 days before | ... | 15 |
| | | | | 1/30/46 | Peripheral vein | Immediately before | ... | .. |
| | | | | | Splenic vein | At operation | ... | .. |
| | | | | | Splenic pedicle | At operation | ... | .. |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | 2/ 8/46 | Peripheral vein | 9 days after | ... | 5 |
| 5 | M | 33 | Parents not studied | 12/ 7/45 | Peripheral vein | 12 days before | ... | 8 |
| | | | | 12/19/45 | Peripheral vein | Immediately before | ... | 5 |
| | | | | | Splenic pedicle | At operation | ... | .. |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | 12/20/45 | Peripheral vein | 1 day after | ... | .. |
| | | | | 12/26/45 | Peripheral vein | 7 days after | ... | .. |
| | | | | 5/ 7/46 | Peripheral vein | 5 mo. after | ... | 5 |
| | | | | 12/ 2/49 | Peripheral vein | 4 yr. after | ... | .. |
| 6 | F | 40 | Daughter of Case 23; sister of Case 24 | 11/25/46 | Peripheral vein | 2 wk. before | 1.6 | 10 |
| | | | | 12/11/46 | Peripheral vein | Immediately before | 1.6 | 10 |
| | | | | | Splenic vein | At operation | 2.4 | 15 |
| | | | | | Splenic pedicle | At operation | ... | 20 |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | | Peripheral vein | 35 min. after | ... | 5 |
| | | | | 12/19/46 | Peripheral vein | 5 days after | 0.8 | 5 |
| | | | | 2/18/47 | Peripheral vein | 2 mo. after | 0.8 | 5 |
| | | | | 5/21/47 | Peripheral vein | 6 mo. after | ... | 5 |
| 7 | F | 8 | Sibling of Case 8 | 10/27/41 | Peripheral vein | Immediately before | 2.1 | 10 |
| | | | | | Splenic pedicle | At operation | | |
| | | | | | First sample | | 2.7 | .. |
| | | | | | Last sample | | ... | .. |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | 10/28/41 | Peripheral vein | 1 day after | 0.5 | 5 |
| | | | | 10/30/41 | Peripheral vein | 3 days after | 0.4 | 4 |
| | | | | 11/14/41 | Peripheral vein | 18 days after | ... | 5 |
| | | | | 12/16/41 | Peripheral vein | 6 wk. after | ... | 5 |
| 8 | M | 18 | Sibling of Case 7 | 1/ 7/41 | Peripheral vein | 1 wk. before | 1.6 | 15 |
| | | | | 1/24/41 | Peripheral vein | 8 days after | 0.4 | 5 |
| | | | | 4/ 4/41 | Peripheral vein | 3 mo. after | ... | 5 |
| 9 | M | 31 | Parents not studied | 1/20/44 | Peripheral vein | Immediately before | ... | 15 |
| | | | | | Splenic vein | At operation | ... | .. |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | 1/21/44 | Peripheral vein | 1 day after | ... | 10 |
| | | | | 1/24/44 | Peripheral vein | 4 days after | ... | 5 |
| | | | | 2/28/44 | Peripheral vein | 5 wk. after | ... | 8 |
| | | | | 1/28/47 | Peripheral vein | 3 yr. after | ... | .. |
| 10 | F | 3½ | Daughter of Case 31 | 3/26/46 | Peripheral vein | 20 days before | ... | 30 |
| | | | | 4/15/46 | Peripheral vein | Immediately before | ... | .. |
| | | | | | Splenic vein | At operation | ... | .. |
| | | | | | Splenic pedicle | At operation | ... | .. |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | 4/16/46 | Peripheral vein | 1 day after | ... | .. |
| | | | | 4/20/46 | Peripheral vein | 5 days after | ... | .. |
| | | | | 11/12/46 | Peripheral vein | 7 mo. after | ... | 7 |

*Thirteen Families (in Three Instances the Parents Were Not Examined)*

| Red Cell Count, Million/ Cu. Mm. | Hemo-globin, Gm./100 Ml. | Hemato-crit Value, % | MCHC, % | Reticulo-cytes, % | Mechanical Fragility of Red Cells % | Osmotic Fragility of Red Cells, Corrected Tonicity, NaCl Gm./100 Ml. Hemolysis, % | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 1 | 2 | 5 | 10 | 25 | 50 | 75 | 90 |
| 1.65 | 4.5 | 13.1 | 34 | ... | .. | 0.71 | 0.69 | 0.67 | 0.65 | 0.57 | 0.49 | 0.47 | 0.45 |
| .... | .... | .... | .. | ... | .. | 0.73 | 0.71 | 0.67 | 0.65 | 0.63 | 0.50 | 0.48 | 0.46 |
| 3.29 | 8.7 | 26.2 | 33 | 2.3 | .. | 0.67 | 0.66 | 0.63 | 0.59 | 0.54 | 0.50 | 0.47 | 0.45 |
| 3.41 | 10.0 | 28.7 | 35 | 0.8 | .. | 0.65 | 0.63 | 0.62 | 0.59 | 0.56 | 0.52 | 0.49 | 0.45 |
| 4.66 | 14.3 | 37.0 | 39 | 2.8 | .. | 0.60 | 0.58 | 0.56 | 0.54 | 0.52 | 0.50 | 0.49 | 0.48 |
| 4.46 | 12.5 | 36.7 | 34 | 1.0 | 7 | 0.52 | 0.49 | 0.46 | 0.45 | 0.43 | 0.42 | 0.39 | 0.36 |
| | | | | (Incubated) | 30 | 0.79 | 0.77 | 0.74 | 0.71 | 0.65 | 0.58 | 0.54 | 0.49 |
| 3.73 | 12.5 | 31.5 | 40 | 6.7 | .. | 0.68 | 0.64 | 0.58 | 0.55 | 0.51 | 0.50 | 0.48 | 0.47 |
| 4.28 | 13.3 | 35.8 | 37 | 1.1 | .. | 0.59 | 0.57 | 0.55 | 0.53 | 0.51 | 0.49 | 0.47 | 0.45 |
| 4.16 | 12.9 | 34.1 | 38 | 0.4 | 9 | 0.51 | 0.49 | 0.46 | 0.45 | 0.44 | 0.43 | 0.40 | 0.35 |
| | | | | (Incubated) | 20 | 0.75 | 0.73 | 0.71 | 0.67 | 0.61 | 0.57 | 0.53 | 0.45 |
| 3.80 | 11.7 | 30.0 | 39 | 6.5 | .. | 0.65 | 0.64 | 0.59 | 0.54 | 0.51 | 0.50 | 0.49 | 0.47 |
| 4.98 | 13.8 | 40.8 | 34 | 0.4 | 7 | 0.50 | 0.48 | 0.46 | 0.45 | 0.44 | 0.43 | 0.39 | 0.37 |
| | | | | (Incubated) | 27 | 0.75 | 0.74 | 0.72 | 0.71 | 0.65 | 0.58 | 0.53 | 0.49 |
| 4.65 | 13.7 | 40.0 | 34 | 0.2 | .. | 0.56 | 0.55 | 0.54 | 0.52 | 0.51 | 0.49 | 0.47 | 0.43 |
| 3.21 | 8.8 | 24.3 | 36 | 16.8 | 19 | 0.67 | 0.55 | 0.53 | 0.49 | 0.44 | 0.41 | 0.39 | 0.36 |
| | | | | (Incubated) | 32 | 0.88 | 0.84 | 0.79 | 0.72 | 0.60 | 0.51 | 0.46 | 0.40 |
| 3.75 | 10.0 | 35.5 | 28 | ... | 7 | 0.70 | 0.59 | 0.54 | 0.47 | 0.43 | 0.42 | 0.40 | 0.38 |
| .... | .... | 35.3 | .. | ... | 15 | 0.68 | 0.65 | 0.60 | 0.56 | 0.46 | 0.43 | 0.39 | 0.35 |
| .... | .... | 40.2 | .. | ... | 19 | 0.73 | 0.69 | 0.65 | 0.62 | 0.58 | 0.45 | 0.41 | 0.37 |
| .... | .... | .... | .. | ... | 32 | 0.73 | 0.71 | 0.69 | 0.67 | 0.62 | 0.57 | 0.46 | 0.42 |
| 4.61 | 13.8 | 37.5 | 37 | 2.5 | 7 | 0.51 | 0.49 | 0.48 | 0.46 | 0.42 | 0.40 | 0.39 | 0.34 |
| | | | | (Incubated) | 34 | 0.82 | 0.81 | 0.79 | 0.77 | 0.73 | 0.59 | 0.49 | 0.43 |
| 2.72 | .... | 28.2 | .. | 7.0 | 10 | 0.63 | 0.58 | 0.52 | 0.48 | 0.45 | 0.43 | 0.42 | 0.39 |
| | | | | (Incubated) | 23 | 0.77 | 0.75 | 0.70 | 0.64 | 0.60 | 0.55 | 0.47 | 0.38 |
| 3.56 | 10.9 | 32.0 | 34 | 1.3 | 11 | 0.59 | 0.52 | 0.48 | 0.46 | 0.44 | 0.42 | 0.41 | 0.38 |
| .... | .... | 29.4 | .. | ... | 16 | 0.63 | 0.61 | 0.57 | 0.53 | 0.45 | 0.41 | 0.39 | 0.35 |
| .... | .... | .... | .. | .... | 23 | 0.72 | 0.68 | 0.63 | 0.60 | 0.54 | 0.45 | 0.42 | 0.37 |
| 4.55 | 11.9 | 38.9 | 31 | 2.6 | 5 | 0.54 | 0.51 | 0.48 | 0.46 | 0.43 | 0.40 | 0.38 | 0.34 |
| .... | .... | 40.0 | .. | ... | 5 | 0.53 | 0.51 | 0.48 | 0.46 | 0.44 | 0.42 | 0.39 | 0.36 |
| 4.42 | 13.7 | 40.2 | 34 | ... | 5 | 0.56 | 0.54 | 0.52 | 0.49 | 0.47 | 0.45 | 0.42 | 0.38 |
| 4.38 | 13.8 | 40.0 | 34 | 1.4 | 8 | 0.66 | 0.64 | 0.61 | 0.59 | 0.56 | 0.53 | 0.51 | 0.44 |
| | | | | (Incubated) | 34 | 0.88 | 0.86 | 0.85 | 0.84 | 0.77 | 0.71 | 0.65 | 0.59 |
| 3.15 | 9.8 | 29.0 | 35 | 10.0 | 11 | 0.68 | 0.64 | 0.57 | 0.50 | 0.48 | 0.45 | 0.43 | 0.37 |
| | | | | (Incubated) | 26 | 0.83 | 0.80 | 0.76 | 0.72 | 0.65 | 0.60 | 0.54 | 0.46 |
| 4.00 | 10.2 | 32.0 | 32 | 1.6 | 6 | 0.68 | 0.53 | 0.51 | 0.50 | 0.49 | 0.46 | 0.43 | 0.41 |
| 6.00 | 16.9 | 48.1 | 35 | 2.1 | 9 | 0.80 | 0.78 | 0.75 | 0.70 | 0.56 | 0.52 | 0.49 | 0.46 |
| 3.96 | 11.7 | 29.4 | 40 | 0.6 | 12 | 0.80 | 0.77 | 0.73 | 0.68 | 0.54 | 0.50 | 0.47 | 0.44 |
| .... | .... | .... | .. | 4.8 | 17 | 0.80 | 0.78 | 0.74 | 0.72 | 0.67 | 0.61 | 0.49 | 0.44 |
| 4.67 | 13.6 | 40.2 | 34 | 3.6 | 9 | 0.70 | 0.68 | 0.62 | 0.54 | 0.50 | 0.48 | 0.46 | 0.42 |
| 4.38 | 12.5 | 36.8 | 34 | 3.9 | 6 | 0.63 | 0.61 | 0.58 | 0.53 | 0.53 | 0.50 | 0.48 | 0.42 |
| 4.67 | 15.0 | 41.1 | 36 | 0.2 | 11 | 0.64 | 0.61 | 0.59 | 0.57 | 0.54 | 0.51 | 0.48 | 0.44 |
| 4.56 | 15.4 | 41.3 | 37 | 2.0 | 12 | 0.60 | 0.58 | 0.56 | 0.54 | 0.50 | 0.49 | 0.47 | 0.44 |
| | | | | (Incubated) | 28 | 0.87 | 0.84 | 0.83 | 0.81 | 0.75 | 0.70 | 0.64 | 0.57 |
| 3.49 | 10.9 | 29.7 | 37 | 6.5 | .. | 0.71 | 0.68 | 0.64 | 0.60 | 0.50 | 0.48 | 0.47 | 0.46 |
| .... | .... | 43.6 | .. | ... | .. | 0.75 | 0.73 | 0.69 | 0.66 | 0.60 | 0.51 | 0.49 | 0.47 |
| .... | .... | 45.2 | .. | ... | .. | 0.83 | 0.79 | 0.75 | 0.63 | 0.68 | 0.60 | 0.51 | 0.49 |
| .... | .... | .... | .. | ... | .. | 0.79 | 0.77 | 0.75 | 0.73 | 0.70 | 0.63 | 0.49 | 0.44 |
| 4.08 | 12.3 | 34.5 | 36 | 10.0 | .. | 0.78 | 0.74 | 0.72 | 0.67 | 0.62 | 0.58 | 0.56 | 0.52 |
| 3.97 | 12.3 | 34.5 | 36 | 9.0 | .. | 0.70 | 0.67 | 0.63 | 0.62 | 0.58 | 0.54 | 0.50 | 0.42 |
| 4.76 | 16.1 | 45.0 | 36 | 0.4 | .. | 0.60 | 0.59 | 0.59 | 0.57 | 0.55 | 0.52 | 0.50 | 0.43 |
| 4.64 | 14.7 | 41.8 | 35 | ... | .. | 0.70 | 0.64 | 0.61 | 0.58 | 0.55 | 0.52 | 0.49 | 0.43 |
| 3.75 | 12.3 | 31.5 | 39 | 2.3 | .. | 0.56 | 0.50 | 0.47 | 0.46 | 0.45 | 0.44 | 0.43 | 0.42 |
| 5.44 | 16.7 | 41.4 | 40 | 0.6 | .. | 0.56 | 0.53 | 0.50 | 0.48 | 0.47 | 0.46 | 0.44 | 0.43 |
| 4.99 | 17.2 | 44.1 | 39 | 0.1 | .. | 0.53 | 0.51 | 0.50 | 0.48 | 0.46 | 0.44 | 0.41 | 0.39 |
| 3.42 | 10.3 | 30.2 | 34 | 11.0 | 13 | 0.73 | 0.71 | 0.68 | 0.66 | 0.62 | 0.50 | 0.47 | 0.44 |
| .... | .... | 31.8 | .. | ... | .. | 0.74 | 0.71 | 0.69 | 0.67 | 0.63 | 0.50 | 0.48 | 0.45 |
| .... | .... | .... | .. | ... | .. | 0.84 | 0.82 | 0.77 | 0.73 | 0.68 | 0.64 | 0.52 | 0.49 |
| 4.02 | 11.1 | 35.7 | 31 | 11.0 | 16 | 0.72 | 0.68 | 0.65 | 0.63 | 0.59 | 0.55 | 0.54 | 0.44 |
| 3.80 | 10.0 | 35.6 | 28 | 12.2 | 10 | 0.65 | 0.63 | 0.60 | 0.58 | 0.54 | 0.51 | 0.49 | 0.44 |
| 5.76 | 17.6 | 50.7 | 35 | 1.3 | .. | 0.59 | 0.57 | 0.54 | 0.52 | 0.50 | 0.46 | 0.42 | 0.34 |
| 6.04 | 17.6 | 50.3 | 35 | 1.6 | 8 | 0.63 | 0.59 | 0.57 | 0.55 | 0.53 | 0.50 | 0.47 | 0.39 |
| | | | | (Incubated) | 18 | 0.83 | 0.81 | 0.78 | 0.72 | 0.65 | 0.61 | 0.55 | 0.45 |
| 4.16 | 11.7 | 34.7 | 34 | 9.7 | 16 | 0.64 | 0.58 | 0.53 | 0.49 | 0.44 | 0.42 | 0.41 | 0.37 |
| | | | | (Incubated) | 52 | 0.81 | 0.80 | 0.77 | 0.74 | 0.69 | 0.60 | 0.53 | 0.43 |
| .... | .... | 34.6 | .. | ... | 18 | 0.59 | 0.55 | 0.47 | 0.45 | 0.43 | 0.42 | 0.39 | 0.35 |
| .... | .... | 37.0 | .. | ... | 20 | 0.61 | 0.60 | 0.57 | 0.52 | 0.42 | 0.41 | 0.39 | 0.35 |
| .... | .... | .... | .. | ... | 41 | 0.70 | 0.68 | 0.64 | 0.61 | 0.58 | 0.46 | 0.43 | 0.40 |
| .... | .... | .... | .. | ... | 31 | 0.74 | 0.66 | 0.61 | 0.59 | 0.56 | 0.51 | 0.42 | 0.38 |
| .... | 10.3 | .... | .. | ... | .. | 0.56 | 0.54 | 0.51 | 0.48 | 0.45 | 0.44 | 0.42 | 0.38 |
| 5.00 | 12.3 | 37.4 | 33 | 2.1 | 13 | 0.56 | 0.54 | 0.51 | 0.49 | 0.46 | 0.44 | 0.40 | 0.38 |
| 4.43 | 15.1 | 41.1 | 37 | 0.4 | 7 | 0.57 | 0.55 | 0.53 | 0.52 | 0.49 | 0.47 | 0.44 | 0.42 |
| | | | | (Incubated) | 20 | 0.80 | 0.78 | 0.74 | 0.72 | 0.64 | 0.58 | 0.55 | 0.49 |

TABLE 4.—*Observations on Thirty-One Patients with Hereditary Spherocytosis,*

| | | | | Blood Sample Tested | | | | |
|---|---|---|---|---|---|---|---|---|
| Case | Sex | Age, Yr. | Familial Incidence | Date | Source | Time Relation to Splenectomy | Serum Bilirubin, Total, Mg./100 Ml. | Icterus Index, Units |
| 11 | F | 3 | Daughter of Case 27 | 11/20/45 | Peripheral vein | 6 wk. before | ... | 10 |
| | | | | 11/26/45 | Peripheral vein | 5 wk. before | 0.9 | .. |
| | | | | 1/ 7/46 | Splenic vein | At operation | ... | .. |
| | | | | | Splenic pedicle | At operation | ... | .. |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | 1/10/46 | Peripheral vein | 3 days after | ... | 10 |
| | | | | 6/11/46 | Peripheral vein | 5 mo. after | ... | 5 |
| | | | | 11/12/46 | Peripheral vein | 10 mo. after | ... | 8 |
| 12 | F | 28 | Daughter of Case 28 | 11/ 7/50 | Peripheral vein | 3 mo. before | 2.2 | 15 |
| | | | | 2/ 1/51 | Peripheral vein | Immediately before | 1.6 | .. |
| | | | | | Splenic pedicle | At operation | ... | .. |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | | Peripheral vein | Immediately after | ... | .. |
| | | | | | Peripheral vein | 8 hr. after | ... | .. |
| | | | | 2/14/51 | Peripheral vein | 48 hr. after | ... | 6 |
| | | | | 3/ 5/51 | Peripheral vein | 13 days after | 0.7 | 5 |
| | | | | 6/ 1/51 | Peripheral vein | 4 mo. after | 0.5 | .. |
| | | | | 9/13/51 | Peripheral vein | 7 mo. after | ... | 7 |
| | | | | 4/ 1/52 | Peripheral vein | 14 mo. after | ... | .. |
| 13 | M | 22 | Son of Case 14; sibling of Cases 15 to 18 | 4/17/47 | Peripheral vein | 12 days before | ... | 20 |
| | | | | 4/29/47 | Peripheral vein | Immediately before | ... | 5 |
| | | | | | Splenic vein | At operation | ... | 5 |
| | | | | | Splenic pedicle | At operation | ... | 15 |
| | | | | | Splenic pulp | At operation | ... | .. |
| | | | | 5/ 2/47 | Peripheral vein | 3 days after | ... | 5 |
| | | | | 6/ 4/47 | Peripheral vein | 1 mo. after | ... | .. |
| | | | | 6/10/48 | Peripheral vein | 13 mo. after | 0.7 | 10 |
| Group II. Patients whose spleens had not been removed | | | | | | | | |
| 14 | F | 57 | Mother of Cases 13, 15-18 | 12/29/49 | Peripheral vein | ..... | 1.0 | 10 |
| 15 | M | 14 | See Case 14 | 12/29/49 | Peripheral vein | ..... | ... | 12 |
| 16 | F | 16 | See Case 14 | 11/18/46 | Peripheral vein | ..... | 1.3 | 8 |
| 17 | F | 20 | See Case 14 | 12/29/49 | Peripheral vein | ..... | ... | 13 |
| 18 | M | 19 | See Case 14 | 11/18/46 | Peripheral vein | ..... | 1.7 | 13 |
| 19 | M | 59 | Father of Cases 20 and 29 | 8/25/48 | Peripheral vein | ..... | ... | 25 |
| 20 | M | 19 | Son of Case 19 | 2/19/47 | Peripheral vein | ..... | 1.0 | 13 |
| 21 | M | 60 | Father of Case 30 | 9/10/47 | Peripheral vein | ..... | 1.3 | 10 |
| 22 | M | 72 | Parents not studied | 9/21/49 | Peripheral vein | ..... | ... | 25 |
| 23 | M | 72 | Father of Cases 6 and 24 | 10/27/46 | Peripheral vein | ..... | 2.0 | 30 |
| 24 | F | 46 | See Case 23 | 10/27/46 | Peripheral vein | ..... | 0.8 | 13 |
| 25 | M | 27 | See Case 1 | 11/20/46 | Peripheral vein | ..... | 0.5 | 5 |
| 26 | M | 45 | Father of Case 4 | 1/24/46 | Peripheral vein | ..... | ... | 8 |
| 27 | M | 37 | Father of Case 11 | 11/26/45 | Peripheral vein | ..... | 1.3 | .. |
| 28 | F | 47 | Mother of Case 12 | 6/ 8/50 | Peripheral vein | ..... | ... | 15 |
| Group III. Patients whose spleen had been removed before observation | | | | | | | | |
| 29 | M | 12 | See Case 19 | 2/25/47 | Peripheral vein | 4 yr. after splenectomy | 1.1 | 8 |
| 30 | M | 24 | Son of Case 21 | 9/10/47 | Peripheral vein | 20 yr. after splenectomy | 0.7 | 8 |
| 31 | M | 40 | Father of Case 10 | 11/12/46 | Peripheral vein | 12 yr. after splenectomy | ... | 18 |

*Representing Thirteen Families (in Three Instances the Parents Were Not Examined)*—Continued

| Red Cell Count, Million/ Cu. Mm. | Hemoglobin, Gm./100 Ml. | Hematocrit Value, % | MCHC, % | Reticulocytes, % | Mechanical Fragility of Red Cells % | Osmotic Fragility of Red Cells, Corrected Tonicity, NaCl Gm./100 Ml. Hemolysis, % 1 | 2 | 5 | 10 | 25 | 50 | 75 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4.09 | 11.6 | 34.8 | 33 | 9.4 | 12 | 0.63 | 0.59 | 0.45 | 0.43 | 0.42 | 0.40 | 0.38 | 0.32 |
| | | | | (Incubated) | 30 | 0.69 | 0.65 | 0.57 | 0.53 | 0.50 | 0.48 | 0.43 | 0.34 |
| 3.65 | 9.2 | 27.9 | 33 | 3.4 | 15 | 0.68 | 0.64 | 0.58 | 0.45 | 0.43 | 0.41 | 0.39 | 0.38 |
| .... | .... | 37.0 | .. | ... | 12 | 0.61 | 0.58 | 0.50 | 0.42 | 0.40 | 0.39 | 0.37 | 0.36 |
| .... | .... | .... | .. | ... | 23 | 0.72 | 0.69 | 0.66 | 0.62 | 0.56 | 0.42 | 0.39 | 0.38 |
| .... | .... | .... | .. | ... | 28 | 0.72 | 0.68 | 0.63 | 0.60 | 0.57 | 0.52 | 0.41 | 0.38 |
| 4.22 | 10.7 | 29.8 | 36 | 5.0 | 8 | 0.56 | 0.53 | 0.49 | 0.46 | 0.43 | 0.40 | 0.38 | 0.35 |
| 6.78 | 12.5 | 35.7 | 35 | 4.7 | 14 | 0.63 | 0.61 | 0.58 | 0.55 | 0.52 | 0.47 | 0.42 | 0.37 |
| 6.07 | 12.5 | 36.6 | 34 | 2.8 | 13 | 0.70 | 0.67 | 0.63 | 0.61 | 0.57 | 0.53 | 0.46 | 0.41 |
| | | | | (Incubated) | 32 | 0.81 | 0.80 | 0.77 | 0.75 | 0.71 | 0.62 | 0.53 | 0.39 |
| 3.68 | 12.6 | 31.0 | 40 | 4.5 | .. | 0.68 | 0.67 | 0.64 | 0.62 | 0.56 | 0.52 | 0.51 | 0.50 |
| | | | | (Incubated) | .. | 0.88 | 0.87 | 0.86 | 0.84 | 0.72 | 0.65 | 0.61 | 0.60 |
| 3.56 | 12.3 | 33.0 | 37 | 6.5 | .. | 0.67 | 0.65 | 0.62 | 0.61 | 0.53 | 0.48 | 0.46 | 0.43 |
| .... | .... | .... | .. | ... | .. | 0.71 | 0.70 | 0.68 | 0.65 | 0.60 | 0.51 | 0.49 | 0.46 |
| .... | .... | .... | .. | ... | .. | 0.77 | 0.72 | 0.69 | 0.65 | 0.62 | 0.56 | 0.52 | 0.47 |
| .... | .... | 34.0 | .. | ... | .. | 0.69 | 0.67 | 0.63 | 0.61 | 0.56 | 0.50 | 0.48 | 0.45 |
| 4.27 | .... | .... | .. | ... | .. | 0.67 | 0.65 | 0.61 | 0.59 | 0.53 | 0.49 | 0.47 | 0.45 |
| .... | .... | .... | .. | ... | .. | 0.62 | 0.60 | 0.56 | 0.54 | 0.50 | 0.47 | 0.45 | 0.44 |
| 4.15 | 13.4 | 35.0 | 38 | 4.3 | .. | 0.58 | 0.57 | 0.55 | 0.53 | 0.50 | 0.48 | 0.47 | 0.45 |
| 4.89 | 14.8 | 39.0 | 38 | 0.8 | .. | 0.60 | 0.58 | 0.56 | 0.53 | 0.50 | 0.49 | 0.47 | 0.44 |
| 4.65 | 14.5 | 39.0 | 37 | 0.7 | .. | 0.57 | 0.56 | 0.53 | 0.52 | 0.50 | 0.48 | 0.46 | 0.43 |
| 4.85 | 14.6 | 40.5 | 36 | 1.9 | .. | 0.67 | 0.56 | 0.54 | 0.52 | 0.51 | 0.49 | 0.48 | 0.45 |
| 4.43 | 13.5 | 38.5 | 35 | 0.8 | .. | 0.87 | 0.85 | 0.83 | 0.79 | 0.71 | 0.64 | 0.58 | 0.48 |
| | | | | (Incubated) | .. | | | | | | | | |
| 4.46 | 14.7 | 38.5 | 38 | 8.2 | 14 | 0.66 | 0.63 | 0.56 | 0.52 | 0.49 | 0.48 | 0.46 | 0.43 |
| | | | | (Incubated) | 31 | 0.85 | 0.83 | 0.80 | 0.74 | 0.69 | 0.65 | 0.62 | 0.54 |
| 2.96 | 10.5 | 30.3 | 35 | 11.0 | 14 | 0.74 | 0.71 | 0.64 | 0.56 | 0.51 | 0.49 | 0.48 | 0.46 |
| 4.43 | 13.5 | 40.2 | 34 | 13.0 | 14 | 0.70 | 0.68 | 0.64 | 0.58 | 0.49 | 0.48 | 0.47 | 0.45 |
| 3.53 | 13.4 | 35.7 | 38 | 8.7 | 21 | 0.80 | 0.78 | 0.75 | 0.72 | 0.66 | 0.52 | 0.50 | 0.48 |
| .... | .... | .... | .. | 6.6 | 22 | 0.79 | 0.77 | 0.73 | 0.71 | 0.68 | 0.63 | 0.50 | 0.48 |
| 2.96 | 10.7 | 30.9 | 35 | 12.0 | 10 | 0.62 | 0.59 | 0.57 | 0.57 | 0.51 | 0.50 | 0.46 | 0.44 |
| 4.72 | 14.1 | 46.0 | 31 | 0.4 | 8 | 0.61 | 0.59 | 0.57 | 0.55 | 0.52 | 0.50 | 0.47 | 0.44 |
| 5.17 | 16.5 | 46.5 | 36 | 0.5 | 11 | 0.66 | 0.64 | 0.61 | 0.59 | 0.56 | 0.54 | 0.51 | 0.46 |
| 5.02 | 15.2 | 43.2 | 35 | 3.1 | 8 | 0.61 | 0.58 | 0.57 | 0.56 | 0.53 | 0.45 | 0.47 | 0.44 |
| | | | | (Incubated) | 29 | 0.86 | 0.84 | 0.82 | 0.81 | 0.78 | 0.72 | 0.65 | 0.56 |
| 4.22 | 12.9 | 36.7 | 35 | 6.1 | 10 | 0.66 | 0.62 | 0.54 | 0.51 | 0.49 | 0.47 | 0.45 | 0.44 |
| | | | | (Incubated) | 37 | 0.89 | 0.84 | 0.83 | 0.82 | 0.76 | 0.68 | 0.62 | 0.54 |
| 3.62 | 11.2 | 30.0 | 37 | 6.8 | 11 | 0.53 | 0.51 | 0.49 | 0.48 | 0.45 | 0.44 | 0.43 | 0.42 |
| | | | | (Incubated) | 32 | 0.78 | 0.76 | 0.73 | 0.71 | 0.66 | 0.61 | 0.57 | 0.53 |
| 3.59 | 11.2 | 31.7 | 35 | 6.6 | 11 | 0.57 | 0.55 | 0.51 | 0.50 | 0.47 | 0.45 | 0.44 | 0.43 |
| | | | | (Incubated) | 34 | 0.86 | 0.85 | 0.82 | 0.74 | 0.73 | 0.66 | 0.61 | 0.55 |
| 4.64 | 15.1 | 40.1 | 38 | 4.6 | 11 | 0.51 | 0.49 | 0.49 | 0.48 | 0.45 | 0.43 | 0.42 | 0.39 |
| | | | | (Incubated) | 29 | 0.79 | 0.76 | 0.72 | 0.69 | 0.62 | 0.57 | 0.49 | 0.41 |
| 3.13 | .... | 26.4 | .. | 5.5 | 10 | 0.67 | 0.61 | 0.54 | 0.52 | 0.49 | 0.47 | 0.44 | 0.41 |
| | | | | (Incubated) | 32 | 0.84 | 0.83 | 0.82 | 0.77 | 0.68 | 0.61 | 0.54 | 0.50 |
| 5.04 | 17.0 | 45.4 | 37 | 5.9 | 12 | 0.59 | 0.57 | 0.53 | 0.51 | 0.50 | 0.47 | 0.46 | 0.43 |
| | | | | (Incubated) | 30 | 0.84 | 0.81 | 0.74 | 0.70 | 0.66 | 0.61 | 0.50 | 0.35 |
| 3.10 | 10.1 | 28.7 | 35 | 3.5 | 11 | 0.65 | 0.54 | 0.48 | 0.47 | 0.44 | 0.42 | 0.41 | 0.38 |
| | | | | (Incubated) | 23 | 0.72 | 0.69 | 0.64 | 0.62 | 0.59 | 0.57 | 0.55 | 0.49 |
| 3.77 | 10.2 | 31.1 | 33 | 12.0 | 11 | 0.68 | 0.66 | 0.58 | 0.50 | 0.48 | 0.47 | 0.45 | 0.41 |
| | | | | (Incubated) | 41 | 0.83 | 0.81 | 0.79 | 0.76 | 0.69 | 0.58 | 0.44 | 0.31 |
| 3.66 | 12.5 | 35.9 | 35 | 13.0 | 10 | 0.55 | 0.47 | 0.46 | 0.45 | 0.44 | 0.42 | 0.40 | 0.31 |
| | | | | (Incubated) | 23 | 0.73 | 0.70 | 0.66 | 0.63 | 0.57 | 0.51 | 0.42 | 0.31 |
| 4.28 | 12.9 | 34.7 | 37 | 8.2 | 12 | 0.50 | 0.47 | 0.46 | 0.44 | 0.43 | 0.42 | 0.40 | 0.32 |
| | | | | (Incubated) | 44 | 0.75 | 0.72 | 0.70 | 0.66 | 0.60 | 0.55 | 0.47 | 0.39 |
| 4.87 | 15.8 | 44.8 | 36 | 2.5 | 10 | 0.51 | 0.50 | 0.49 | 0.48 | 0.46 | 0.43 | 0.42 | 0.41 |
| | | | | (Incubated) | 17 | 0.79 | 0.74 | 0.67 | 0.65 | 0.62 | 0.59 | 0.57 | 0.50 |
| 4.03 | 13.8 | 44.8 | 31 | 0.8 | 4 | 0.42 | 0.41 | 0.40 | 0.39 | 0.37 | 0.36 | 0.35 | 0.33 |
| | | | | (Incubated) | 29 | 0.72 | 0.67 | 0.63 | 0.57 | 0.51 | 0.46 | 0.41 | 0.32 |
| 4.68 | 14.2 | 42.8 | 33 | 6.3 | 8 | 0.54 | 0.51 | 0.48 | 0.46 | 0.43 | 0.42 | 0.41 | 0.39 |
| | | | | (Incubated) | 22 | 0.67 | 0.59 | 0.56 | 0.55 | 0.53 | 0.51 | 0.49 | 0.46 |
| 3.31 | 10.6 | 27.0 | 39 | 14.0 | .. | 0.72 | 0.70 | 0.67 | 0.65 | 0.62 | 0.53 | 0.45 | 0.44 |
| | | | | (Incubated) | .. | 0.96 | 0.94 | 0.92 | 0.90 | 0.87 | 0.79 | 0.63 | 0.54 |
| 4.98 | 14.3 | 39.0 | 37 | 0.4 | 9 | 0.55 | 0.54 | 0.52 | 0.51 | 0.49 | 0.47 | 0.45 | 0.42 |
| | | | | (Incubated) | 19 | 0.81 | 0.80 | 0.76 | 0.72 | 0.65 | 0.60 | 0.56 | 0.52 |
| 4.53 | 14.8 | 42.9 | 35 | 0.8 | 14 | 0.63 | 0.60 | 0.57 | 0.55 | 0.52 | 0.50 | 0.48 | 0.44 |
| | | | | (Incubated) | 28 | 0.82 | 0.79 | 0.78 | 0.76 | 0.73 | 0.69 | 0.64 | 0.59 |
| 5.78 | 15.6 | 47.0 | 33 | 0.4 | 7 | 0.60 | 0.57 | 0.56 | 0.54 | 0.51 | 0.49 | 0.45 | 0.40 |
| | | | | (Incubated) | 21 | 0.83 | 0.80 | 0.78 | 0.75 | 0.68 | 0.61 | 0.56 | 0.50 |

TABLE 5.—*Observations on Patients Who Had Splenectomy for Conditions Other Than Hereditary Spherocytosis*

| Case | Sex | Age, Yr. | Diagnosis | Date | Source | Time Relation to Splenectomy | Hematocrit Value, % | Mechanical Fragility of Red Cells, % | Osmotic Fragility of Red Cells, Corrected Tonicity, NaCl Gm./100 Ml. Hemolysis, % | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 1 | 2 | 5 | 10 | 25 | 50 | 75 | 90 |
| 32 | F | 22 | Idiopathic thrombocytopenic purpura | 3/ 6/43 | Peripheral vein | Immediately before | 44.0 | ... | 0.47 | 0.46 | 0.45 | 0.44 | 0.43 | 0.42 | 0.40 | 0.39 |
| | | | | | Splenic pulp | At operation | .... | ... | 0.49 | 0.47 | 0.46 | 0.44 | 0.43 | 0.42 | 0.40 | 0.37 |
| 33 | F | 28 | Idiopathic thrombocytopenic purpura | 5/13/43 | Peripheral vein | Immediately before | 39.2 | ... | 0.51 | 0.50 | 0.49 | 0.49 | 0.47 | 0.46 | 0.45 | 0.42 |
| | | | | | Splenic pulp | At operation | .... | ... | 0.49 | 0.48 | 0.46 | 0.45 | 0.44 | 0.43 | 0.42 | 0.40 |
| 34 | F | .. | Idiopathic thrombocytopenic purpura | 11/10/43 | Peripheral vein | Immediately before | 40.0 | ... | 0.50 | 0.49 | 0.47 | 0.46 | 0.44 | 0.42 | 0.41 | 0.39 |
| | | | | | Splenic pulp | At operation | .... | ... | 0.54 | 0.52 | 0.49 | 0.48 | 0.47 | 0.45 | 0.43 | 0.41 |
| 35 | M | 26 | Idiopathic thrombocytopenic purpura | 9/23/47 | Peripheral vein | Immediately before | 47.7 | 1.8 | 0.48 | 0.47 | 0.45 | 0.44 | 0.43 | 0.41 | 0.40 | 0.37 |
| | | | | | Splenic pulp | At operation | .... | ... | 0.52 | 0.51 | 0.49 | 0.46 | 0.43 | 0.41 | 0.40 | 0.37 |
| 36 | F | 28 | Idiopathic thrombocytopenic purpura | 7/ 1/48 | Peripheral vein | Before operation | 38.3 | 1.7 | 0.46 | 0.45 | 0.44 | 0.43 | 0.42 | 0.40 | 0.38 | 0.35 |
| | | | | | Splenic pulp | At operation | .... | ... | 0.50 | 0.47 | 0.44 | 0.41 | 0.39 | 0.37 | 0.36 | 0.32 |
| 37 | M | 24 | Idiopathic thrombocytopenic purpura | 4/ 4/49 | Peripheral vein | Immediately before | 47.9 | 2.2 | 0.50 | 0.48 | 0.46 | 0.45 | 0.44 | 0.42 | 0.40 | 0.37 |
| | | | | | Splenic pulp | At operation | .... | 7.3 | 0.54 | 0.52 | 0.48 | 0.44 | 0.42 | 0.40 | 0.37 | 0.36 |
| 38 | F | 55 | Pancytopenia | 3/30/46 | Peripheral vein | Immediately before | 40.5* | 4.2 | 0.51 | 0.49 | 0.46 | 0.43 | 0.41 | 0.40 | 0.36 | 0.35 |
| | | | | | Splenic pulp | At operation | .... | 6.0 | 0.59 | 0.57 | 0.52 | 0.46 | 0.42 | 0.40 | 0.38 | 0.35 |
| 39 | M | .. | Pancytopenia | 3/ 6/46 | Peripheral vein | Immediately before | 39.4* | 4.7 | 0.47 | 0.46 | 0.44 | 0.43 | 0.42 | 0.40 | 0.38 | 0.36 |
| | | | | | Splenic pulp | At operation | .... | 8.0 | 0.48 | 0.47 | 0.45 | 0.43 | 0.38 | 0.37 | 0.35 | 0.33 |

* Patients previously transfused with large amounts of blood (see Table 8).

of 1%, 2%, 5%, 10%, 25%, 50%, 75%, and 90%, as shown in Tables 1, 4, 5, and 6. From the summation curves of hemolysis, data were also extrapolated, as shown in columns (6), (7), and (8) of Table 1, to produce the distribution curves of osmotic hemolysis in Figures 1, 3, 5 to 10, and 12,

TABLE 6.—*Osmotic Fragility of Red Cells from Peripheral Blood Before and After Incubation at 37.5 C for Twenty-Four Hours of Samples from Patients with Hereditary Spherocytosis and from Normal Subjects* *

| | Corrected Tonicity, NaCl Gm./100 Ml., Mean ± σ | | |
|---|---|---|---|
| | | Hereditary Spherocytosis | |
| Hemolysis, % | Normal Persons (31 Subjects) | Spleen Not Removed (22 Cases) | Spleen Removed (13 Cases) |
| Unincubated | | | |
| 1 | 0.47±0.027 | 0.60±0.080 | 0.58±0.06 |
| 2 | 0.46±0.027 | 0.56±0.07 | 0.56±0.06 |
| 5 | 0.44±0.025 | 0.52±0.06 | 0.53±0.06 |
| 10 | 0.43±0.026 | 0.49±0.06 | 0.52±0.05 |
| 25 | 0.42±0.024 | 0.47±0.05 | 0.49±0.05 |
| 50 | 0.40±0.022 | 0.44±0.04 | 0.47±0.04 |
| 75 | 0.38±0.020 | 0.42±0.04 | 0.44±0.04 |
| 90 | 0.36±0.025 | 0.37±0.05 | 0.40±0.04 |
| Incubated | | | |
| 1 | 0.59±0.030 | 0.80±0.07 | 0.82±0.04 |
| 2 | 0.57±0.029 | 0.77±0.08 | 0.80±0.04 |
| 5 | 0.55±0.028 | 0.74±0.09 | 0.78±0.04 |
| 10 | 0.54±0.025 | 0.71±0.10 | 0.75±0.04 |
| 25 | 0.51±0.027 | 0.65±0.09 | 0.69±0.05 |
| 50 | 0.49±0.028 | 0.59±0.08 | 0.62±0.05 |
| 75 | 0.46±0.028 | 0.53±0.08 | 0.56±0.05 |
| 90 | 0.42±0.035 | 0.42±0.09 | 0.50±0.06 |

* These data are plotted in Figures 2 and 3 and show the hemolysis of red cells (mean ± 1 S. D.) in hypotonic salt solutions. Observations were made on 22 patients with hereditary spherocytosis whose spleens had not been removed and on 13 patients after splenectomy. See Table 4. Those not included of the 28 cases (6 cases) did not have observations on incubation before splenectomy.

which were prepared in the following manner: From a carefully drawn "smooth" summation curve, the hemolysis in per cent was read at intervals equivalent to a concentration of sodium chloride of 0.02 gm/100 ml., and the increment of hemolysis for graduations of tonicity was calculated by subtraction, as shown in Table 1. Percentage hemolysis was then plotted on the ordinate in relation to the corrected tonicity on the abscissa, with use of a negative logarithmic scale, i. e., —log *Tc*, or 1/log *Tc*, where *Tc* is the corrected tonicity of the saline solution.* This method of graphing produced a distribution curve of hemolysis.

Studies were made of normal red cells and red cells from patients with hereditary spherocytosis to determine the mean hemolysis, and its variations, occurring at various levels of tonicity. To accomplish this, data were extrapolated as demonstrated in columns (4) and (5) in Table 1 and analyzed by standard statistical procedures, as reported in Table 6. Summation curves plotted from these data are shown in Figure 2. From these summation curves, in turn, data were obtained by extrapolation for the preparation of distribution curves, which are shown in Figures 1 and 3.

### MECHANICAL FRAGILITY

The susceptibility of red cells to hemolysis by "trauma" was measured by a modification of the mechanical fragility test described by Shen, Castle, and Fleming † as follows: A sample of fresh venous blood containing dry oxalate mixture was adjusted to a hematocrit value of 35% by the addition or removal of plasma. One portion of 0.5 ml. of the adjusted sample was transferred to a Pyrex Erlenmeyer flask of 50 ml. capacity containing 10 glass beads of uniform size, 4 mm. in diameter. This flask was stoppered and clamped to the periphery of a disc 13 in. in diameter, which was rotated on a horizontal axis at 30 rpm for 90 minutes at a temperature of 37 C. Plasma was then removed from the specimen and from a control specimen which had been kept at 37 C but had not been rotated. The concen-

* Selection of the negative logarithm scale for the plotting of tonicity was based on the observation that distribution curves prepared in this manner from normal subjects are more nearly symmetrical than if plotted on Cartesian coordinates. Further observations concerning the relationship between tonicity, cell volumes, and hemolysis will be reported in a separate communication.

† References 17, 30, and 31.

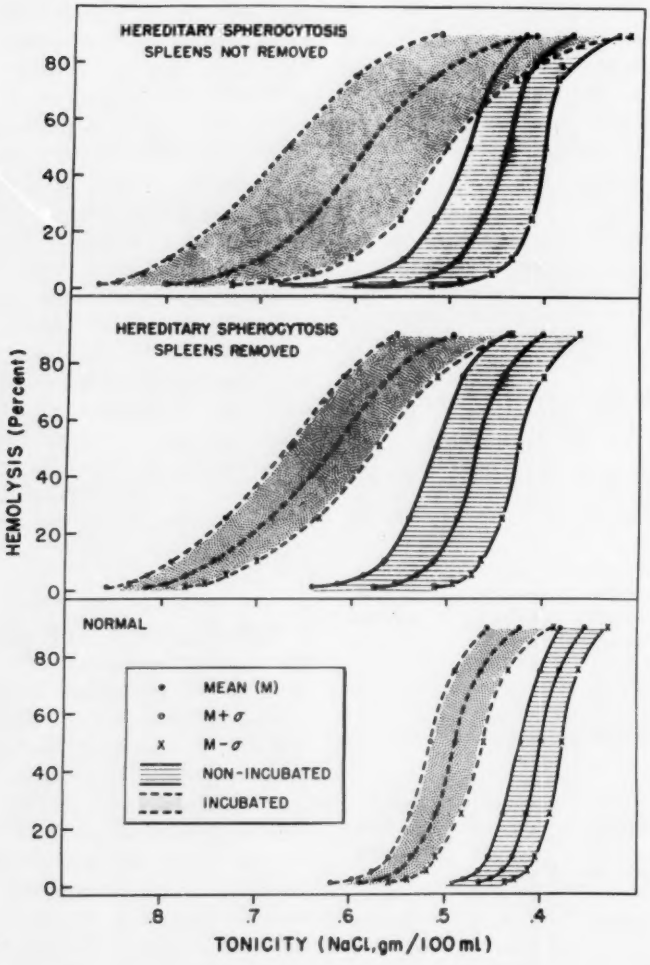


Fig. 2.—Osmotic fragility of red cells from defibrinated peripheral blood before and after incubation at 37 C for 24 hours of samples from patients with hereditary spherocytosis and from 31 normal subjects. These summation curves show the total percentage of red cells hemolyzed in hypotonic salt solutions, plotted in relation to the mean tonicity (±1 S. D.) at which hemolysis occurred. The data are shown in Table 6. Observations were made on 22 patients with hereditary spherocytosis whose spleens had not been removed and on 13 patients after splenectomy. The corresponding data are plotted in the form of distribution curves in Figure 1 for normal control subjects and in Figure 3 for the patients with hereditary spherocytosis.

tration of hemoglobin was measured in both samples of plasma and in the adjusted sample of whole blood, a portion of which had been hemolyzed in distilled water. By dividing the difference between the concentrations of extracellular hemoglobin in the rotated and unrotated specimens by the total hemoglobin concentration a value was obtained which, multiplied by 100, indicated the proportion of red cells hemolyzed by trauma. This was reported as the mechanical fragility. Tests on normal unincubated blood showed a mechanical fragility percentage of 3±1, as indicated in Table 7.

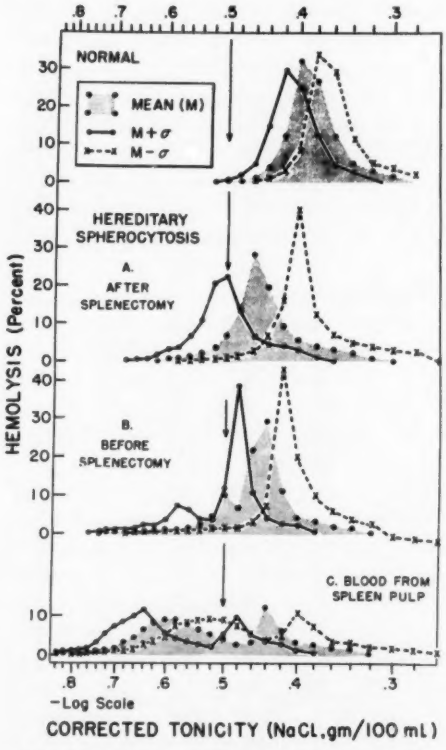


Fig. 3.—Osmotic fragility of red cells in hereditary spherocytosis, as observed in venous blood before and after splenectomy and in the spleen, compared with normal cells. These distribution curves show the percentage of red cells hemolyzed in hypotonic salt solutions and are plotted in relation to the mean tonicity, ±1 S. D., at which hemolysis occurred. An arbitrary guide line (arrow) is drawn vertically at a tonicity of 0.5 gm. NaCl/100 ml. Compare with Figure 1. The subjects studied were as follows: 31 normal subjects, peripheral blood; 13 patients with hereditary spherocytosis (see Table 4); *A*, peripheral blood after splenectomy; *B*, peripheral blood before splenectomy; *C*, red cells from the splenic pulp in nine of the patients shown in *A* and *B*.

TABLE 7.—*Mechanical Fragility of Red Cells Before and After Incubation in Patients with Hereditary Spherocytosis and in Normal Subjects*

| | Hemolysis, %, Mean ± σ | | |
|---|---|---|---|
| | | Hereditary Spherocytosis | |
| | Normal (31 Cases) | Spleen Not Removed (19 Cases) | Spleen Removed (12 Cases) |
| Unincubated | 3±1 | 11±3 | 9±3 |
| Incubated | 11±4 | 32±8 | 27±6 |

### STERILE INCUBATION OF DEFIBRINATED BLOOD

Samples of sterile defibrinated venous blood from patients with hereditary spherocytosis and from normal subjects were incubated for 24 hours in an air incubator at 37 C at pH 7.2 to 7.4. The hematocrit value and the osmotic and mechanical fragility of each sample were measured before and after incubation, the pH having been stabilized as described above. It was demonstrated that oxygen, carbon dioxide, or nitrogen affected osmotic fragility only to the extent that the presence or absence of these gases altered the pH of the red cell. The samples were not mixed during incubation, and no glucose was added.

### EXPERIMENTAL PROCEDURES AT THE TIME OF SPLENECTOMY

Observations were made on blood samples obtained from the spleen in nine cases of hereditary spherocytosis and in eight cases of other types of hemolytic anemia. At the time of operation ‡ a sample of blood was obtained from the splenic vein and introduced into dry oxalate mixture. Immediately after removal of the spleen the blood draining from the splenic pedicle (splenic drip) was collected in dry oxalate mixture or was defibrinated. The spleen was then cut into small pieces, minced in a dry, clean, domestic meat grinder, and transferred to a clean, dry, glass funnel containing a gauze filter (splenic pulp). The pH of the freshly ground spleen pulp was observed over a range of 6.3 to 7.0. From 20 to 60 ml. of homologous serum was added to the splenic pulp in the funnel, and the fluid portion was allowed to drain into a collecting flask. This material was immediately subjected to centrifugation, followed by removal of the serum and tissue cells. Hemolysis was observed regularly in the supernatant for one or two washings. The red cells layered at the bottom of the tube were then suspended in an equal volume of serum, equilibrated

‡ The splenic artery was always ligated before the splenic vein; the spleen was usually contracted before being removed.

with room air in a large Erlenmeyer flask for four to five minutes, washed a second time in serum, and finally resuspended in fresh serum, the hematocrit value being adjusted to approximately 35%. This mixture was reequilibrated, and the pH was stabilized within the range of 7.2 to 7.4 in preparation for the tests of osmotic and mechanical fragility.

### Experimental Transfusions and Donor Cell Counts

Five patients with hereditary spherocytosis and two patients with nonhemolytic anemia were transfused until there was a substantial proportion of normal red cells in their circulating blood, as measured quantitatively by the differential agglutination principles as described for the ABO system by Ashby[32] and for the MN system by Wiener.[33] Donor cell counts were performed according to the procedure described by Ebert and Emerson,[11] employing lyophilized rabbit antiserum § as the agglutinating reagent and the test-tube centrifuge technique as the method of agglutination. This method has been used by Young and co-workers.[34] Three Group A patients were transfused preoperatively with Group O donor blood from which most of the plasma had been removed, or to which Witebsky substance had been added, in order to avoid damage to the recipient's cells by incompatible isoantibodies (See Table 9). The concentration of Group O donor cells in blood obtained from

§ The A, B, and M antisera used in this study were furnished by Lederle Laboratories Division, American Cyanamid Company. The sera were harvested from immune rabbits, lyophilized, and used in powder form.

Table 8.—*Comparison of the Distribution of Normal Donor Red Cells and Patient's Cells in the Peripheral Blood and in the Spleen in Hereditary Spherocytosis and Idiopathic Pancytopenia* *

| Case No. | Per Cent of Patient's Red Cells | |
|---|---|---|
| | Peripheral Blood | Splenic Pulp |
| **Hereditary spherocytosis** | | |
| 4 | 46 | 89 |
| 5 | 48 | 76 |
| 6 | 50 | 88 |
| 12 | 91 | 98 |
| 13 | 80 | 96 |
| **Idiopathic pancytopenia** | | |
| 38 | 26 | 27 |
| 39 | 50 | 53 |

* In hereditary spherocytosis there is a disproportionately high concentration of the patient's red cells in the spleen, indicating their selective retention.

Table 9.—*Survival of Normal Donor Red Cells Transfused into Five Patients with Hereditary Spherocytosis* *

| Case | Blood Group | Day | Red Cell Count, Million/Cu. Mm. | | Reticulocytes, % | Icterus Index, Units |
|---|---|---|---|---|---|---|
| | | | Donor | Patient | | |
| 4 | A | 0 | 0 | 3.21 | 16.8 | 15 |
| | | Transfusion 250 ml. red cells, Group O | | | | |
| | | 1 | 2.18 | 3.27 | 1.7 | 12 |
| | | 4 | 2.25 | 2.42 | .... | .. |
| | | 9 | 1.76 | 1.99 | .... | .. |
| | | 9 | Splenectomy (1/30/46) | | | |
| | | 14 | 1.63 | 3.05 | 2.5 | 5 |
| | | 18 | 1.15 | 3.46 | .... | .. |
| 5 | A | 0 | 0 | 2.91 | 8 | 8 |
| | | Transfusion 600 ml. red cells, Group O (plasma removed) | | | | |
| | | 1 | 1.00 | 2.76 | 1.7 | 8 |
| | | 6 | 0.86 | 2.39 | 1.6 | 5 |
| | | 6 | Transfusion 500 ml. red cells, Group O (plasma removed) | | | |
| | | 8 | 1.62 | 1.89 | 0.8 | 8 |
| | | 11 | 1.43 | 1.81 | 1.4 | 5 |
| | | 12 | 1.42 | 1.74 | 1.3 | 5 |
| | | 12 | Splenectomy (12/19/45) | | | |
| | | 13 | Transfusion 250 ml., Group A | | | |
| | | 26 | 1.12 | 3.38 | .... | 3 |
| | | 39 | 0.82 | 3.70 | 1.3 | 5 |
| | | 47 | 0.59 | 4.49 | .... | .. |
| | | 71 | 0.27 | 4.57 | .... | 5 |
| 6 | A | 0 | 0 | 3.15 | 10 | 10 |
| | | Transfusion 800 ml. red cells, Group O (plasma removed) | | | | |
| | | 1 | 2.10 | 2.21 | .... | 30 |
| | | 7 | 2.14 | 1.83 | 5.4 | 10 |
| | | 16 | 2.00 | 2.00 | 1.6 | 10 |
| | | 16 | Splenectomy (12/11/46) | | | |
| | | 16 | 2.00 | 2.67 | 3.6 | 5 |
| | | 17 | 1.98 | 2.71 | 4.8 | 5 |
| | | 21 | 1.66 | 2.72 | 3.9 | 5 |
| | | 25 | 1.34 | 2.84 | 1.6 | 3 |
| | | 47 | 1.12 | 3.38 | .... | .. |
| | | 86 | 0.37 | 4.30 | 0.2 | 5 |
| 12 | OMN | 0 | 0 | 3.73 | .... | .. |
| | | Transfused 250 ml. red cells, Group ON | | | | |
| | | 1 | 0.68 | 3.82 | .... | .. |
| | | 2 | 0.68 | 3.32 | .... | .. |
| | | 14 | 0.55 | 3.79 | .... | .. |
| | | 28 | 0.46 | 3.54 | .... | .. |
| | | 41 | 0.40 | 3.43 | 6.5 | 8 |
| | | 54 | 0.31 | 3.24 | .... | .. |
| | | 54 | Splenectomy (2/12/51) | | | |
| | | 54 | 0.30 | 3.97 | .... | .. |
| | | 56 | 0.26 | 3.89 | .... | 10 |
| | | 59 | 0.26 | 3.94 | .... | .. |
| | | 75 | 0.18 | 4.71 | .... | .. |
| | | 120 | 0.08 | 4.85 | .... | .. |
| 13 | OM | 0 | 0 | 4.45 | 6.7 | 15 |
| | | Transfused 500 ml. red cells, Group ON | | | | |
| | | 1 | 1.06 | 3.87 | 4.7 | 20 |
| | | Transfused 500 ml. red cells, Group ON | | | | |
| | | 2 | 1.40 | 3.25 | 3.4 | 15 |
| | | 4 | 1.39 | 3.36 | 3.0 | 20 |
| | | 6 | 1.30 | 2.81 | 1.5 | 22 |
| | | 8 | 1.06 | 2.91 | 6.6 | 17 |
| | | 12 | 0.66 | 2.83 | 11 | 5 |
| | | 12 | Splenectomy (4/29/47) | | | |
| | | 18 | 0.38 | 2.52 | 11 | 5 |
| | | 18 | 0.22 | 3.12 | 5.9 | .. |
| | | 33 | 0.12 | 4.36 | 0.4 | 4 |

* Following transfusion of normal donor red cells, there was a significant decrease in the number of patients' cells in the four cases who received enough donor blood to exceed a red cell count of 1,400,000/cu. mm. In Cases 6 and 13 there was a rapid decrease in the patients' red cells within 24 hours of transfusion (see Fig. 11 for Case 6). Reticulocytes decreased after transfusion. The only significant increase in icterus occurred in Case 6, immediately after transfusion.

these recipients following transfusion was determined on the basis of the number of cells which failed to be agglutinated by anti-A serum, in proportion to the total number of cells in the sample. Four patients belonging to blood group OM or OMN received transfusions from group ON donors, the counting of donor cells in these recipients being performed with the use of anti-M serum.

Unagglutinable cells were counted in specimens of defibrinated or oxalated blood from the antecubital and splenic veins and in samples of red cells which had been separated from the spleen pulp, then washed and suspended in normal serum. Cell suspensions in saline were prepared by mixing 0.1 ml. samples of undiluted blood with 20 ml. of a solution containing 1.0 gm. of NaCl per 100 ml. and by diluting spleen samples with volumes of 1% saline sufficient to produce suspensions containing between 20,000 and 50,000 red cells per cu. mm. One 0.1 ml. aliquot sample of each suspension was placed in a test tube measuring 12×75 mm. containing approximately 10 mg. of dried A, B, or M antiserum. A second aliquot sample was transferred to a standard hemocytometer, and a cell count was performed. The antiserum having dissolved completely, the first sample was centrifuged for one minute at 1000 rpm, after which the sediment was resuspended by 30 to 40 vigorous taps on the tube. This was then allowed to stand for five minutes at room temperature. Finally, an aliquot sample of this suspension, containing a mixture of agglutinated and free cells, was transferred to a hemocytometer and the unagglutinated cells were counted. All counts were performed in duplicate. The number of free cells in proportion to the total number of cells in the sample containing no antiserum, multiplied by 100, represented the percentage of donor cells in the population. The absolute number of donor cells in specimens of whole blood diluted with 200 volumes of saline was based on the following formula:

$$\text{RBC/cu. mm.} = \frac{\text{number of free cells counted}}{\text{area of chamber observed (sq. mm.)}} \times 2000$$

### Differential Osmotic Fragility Determinations in Samples Containing a Mixture of Cell Populations

The agglutinative potency, i. e., the avidity, of A and M antiserums was observed to be undiminished in hypotonic solutions of sodium chloride, and it was discovered that reliable counts of unagglutinable cells could be performed on suspensions, the tonicity of which was sufficiently low to produce almost complete hemolysis. The validity of these data having been established, selective agglutination counts were carried out in conjunction with quantitative osmotic fragility tests on samples, artificially prepared, which contained predetermined proportions of Group O cells and cells belonging to Groups A, B, or AB and on various mixtures of Group N and Group M or MN cells. The results of these tests indicated that it is possible to characterize, with reasonable accuracy, the osmotic fragility of individual cell populations in samples representing a mixture of populations which differ with respect to blood group specificity, providing their difference is based on the presence or absence of a strong antigen, such as $A_1$ or M. Details of the procedure, as it was applied in the course of this study, are as follows:

Aliquot portions, 0.3 ml. in volume, of oxalated blood from the transfusion recipient under investigation were added to 3.0 ml. samples of saline, the tonicity of which varied from 1.0 to 0 gm. NaCl per 100 ml., as specified earlier in relation to osmotic fragility tests. A uniform suspension having been obtained by mechanical agitation, a 1.0 ml. sample of each mixture was transferred to an empty tube in preparation for selective agglutination counting. The red cells in the remainder of the specimen having been sedimented by centrifugation, the supernatant solution was removed by decanting, diluted with 20 volumes of distilled water, and tested for hemoglobin in a photoelectric colorimeter, as a basis for computing the

percentage hemolysis that had occurred at each level of tonicity.

Next, each of the 1.0 ml. aliquot samples was diluted with 1% saline solution, the volume of diluent varying from 9.0 ml. for suspensions showing no osmotic hemolysis to 1.0 ml. for those in which the majority of cells were hemolyzed, as demonstrated by colorimetric testing of the aliquot sample. Two drops (approximately 0.1 ml.) of each diluted suspension were then transferred to a 10×75 mm. test tube containing approximately 10 mg. of lyophilized rabbit serum containing antibodies specific for the blood group antigen A, B, or M, depending upon which of these antigens was possessed by the transfusion recipient and was not contained in the donor blood. After centrifugation and resuspension of the cells, in the manner described in the preceding section, the unagglutinated cells were counted. In order to compensate for the paucity of cells in the more hypotonic and hemolyzed samples, and thereby reduce the field counting errors to a minimum, the area counted was increased to as much as 18 sq. mm., which represents 1.8 cu. mm. of cell suspension containing 0.08 cu. mm. of the original blood sample, i. e., approximately 800 times as much blood as is customarily examined in a standard red cell count.

Steps in the calculation of differential osmotic fragility data, and the type of data obtained, are illustrated in Table 3. This method of investigation was employed in studying the peripheral and splenic blood of patients with hereditary spherocytosis who had received transfusions in the manner which has been described.

## THE MECHANISM OF INCREASED HEMOLYSIS IN HEREDITARY SPHEROCYTOSIS (CONGENITAL HEMOLYTIC JAUNDICE) AS RELATED TO THE FUNCTIONS OF THE SPLEEN

### 1. Characteristics of Red Cells in the Peripheral Blood and Spleen Pulp in Control Cases

In order to establish a basis on which to evaluate the findings obtained in cases of hereditary spherocytosis, six patients without evidence of hemolytic anemia, including four with idiopathic thrombocytopenia and two with pancytopenia, were studied at the time of splenectomy.

*Experimental Data.*—As shown in Table 5 and Figure 1, the mean osmotic fragility of red cells, both in the peripheral blood and from the spleens of these patients, was approximately normal. Blood from the spleen pulp in Cases 34, 35, 37, and 38 contained a small proportion of erythrocytes with an increased osmotic fragility, as evidenced by small "trails" on the fragility curves, which were otherwise symmetrical and normal with respect to position and configuration. The mechanical fragility of red cells from the spleen, as compared to those from the peripheral blood, was moderately increased in Cases 37, 38, and 39, the only instances in which these data are available.

### 2. Characteristics of Red Cells in Cases of Hereditary Spherocytosis After Splenectomy

Red cells which have never been exposed to the influence of the spleen might be expected to represent, with relatively little distortion, the type of erythrocyte produced by the bone marrow in hereditary spherocytosis. Accordingly, it should be possible to characterize the inherent properties of the red cell in this disease more precisely after, rather than before, splenectomy.

*Experimental Study.* — Sixteen patients with hereditary spherocytosis were studied after splenectomy, the time interval after operation extending for periods as long as 6 years in Cases 1 to 13 and 20 years in Cases 29 to 31. These data, including erythrocyte and reticulocyte counts, concentrations of hemoglobin and bilirubin or icterus index, the osmotic and mechanical fragility of red cells before and after incubation, and the survival of transfused donor cells, are recorded in Tables 4, 6, 7, and 9 and in Figures 3 to 10.

As shown in Table 4, splenectomy in Cases 1 to 13 was followed promptly by restoration of the erythrocyte and reticulo-

cyte || counts, the hemoglobin concentration, and the icterus index to approximately normal levels, which were maintained thereafter as long as these patients were observed. Approximately normal values were also obtained in Cases 29 to 31, whose spleens had been removed many years prior to this study. In most of these cases the hematocrit reading was disproportionately low in relation to the concentration of hemoglobin in whole blood, indicating that the values for mean corpuscular hemoglobin concentration were elevated. This observation was apparently unrelated to the presence or absence of the spleen or to the time of splenectomy.

The curves of the osmotic fragility test obtained one week or longer after splenectomy were monophasic and approximately symmetric, as demonstrated by individual cases in Figures 5 to 10 and by the average findings in 13 cases, illustrated in Figure 3*A*. The absolute level of osmotic fragility continued to be increased above normal after splenectomy, with about the same range of tonicity before and after splenectomy, as indicated in Tables 4 and 6 and Figures 2 and 3. Incubation at 37 C resulted in a marked increase in osmotic fragility, which invariably exceeded the change produced in normal red cells. As shown in Tables 4 and 6 and in Figure 2, the effect of incubation was similar in magnitude before and after splenectomy and was independent of such factors as the time interval after operation or the concentration of other blood constituents, including reticulocytes, leucocytes, and bilirubin.

The mechanical fragility of red cells before and after incubation was significantly increased above that of normal blood, to a degree which was comparable to that observed before splenectomy. These data are shown in Tables 4 and 7.

|| In Case 11 the reticulocytes remained elevated for 5 and 10 months after splenectomy, associated with striking microcytosis and extreme increase in osmotic fragility. There was only slight icterus. These data suggest continuing moderate blood destruction, but the hemoglobin returned to 12.5 gm/100 ml.

It was observed that blood from the same normal donor ¶ was destroyed by the patient (Case 12) at the same normal rate both before and after splenectomy, as shown in Figure 4. The survival of red cells from Case 12 transfused into the same normal subject was measured before and after splenectomy # of Case 12, as shown in Figure 4 and Table 9. In each instance the patient's cells were destroyed rapidly, as an exponential function of time, and at the same rate despite restoration to normal after splenectomy of the patient's red cell count, reticulocyte count, and icterus index. However, the survival was approximately normal when red cells from Case 12, obtained after splenectomy,* were transfused into a normal subject whose spleen had been removed nine months previously because of traumatic rupture.

*Interpretation.*—As a result of splenectomy in hereditary spherocytosis levels of hemoglobin, reticulocytes, and bilirubin become normal, indicating that red cell destruction and red cell production proceed at a normal rate in the absence of the spleen. Nevertheless, the red cells continue to exhibit abnormal osmotic and mechanical fragilities, increased mean corpuscular hemoglobin concentration, and increased susceptibility to destruction when transfused into a normal subject.

The curve of osmotic fragility characteristic of patients with hereditary spherocytosis after splenectomy, in common with normal subjects, is monophasic and symmetric. Its span, however, is greatly increased, owing to the fact that a large component of these cell populations is spheroidal and therefore abnormally susceptible to hypotonic hemolysis. Since the distribution curve of the osmotic fragility is

¶ The donor was ON Rh positive (D).

# Cells were obtained before splenectomy on Dec. 20, 1950, and after splenectomy on June 1, 1951. The patient was blood group OM Rh positive (D); the recipient, BM Rh positive (D).

* Cells were obtained in June, 1953. The normal recipient whose spleen had been removed was blood Group A.

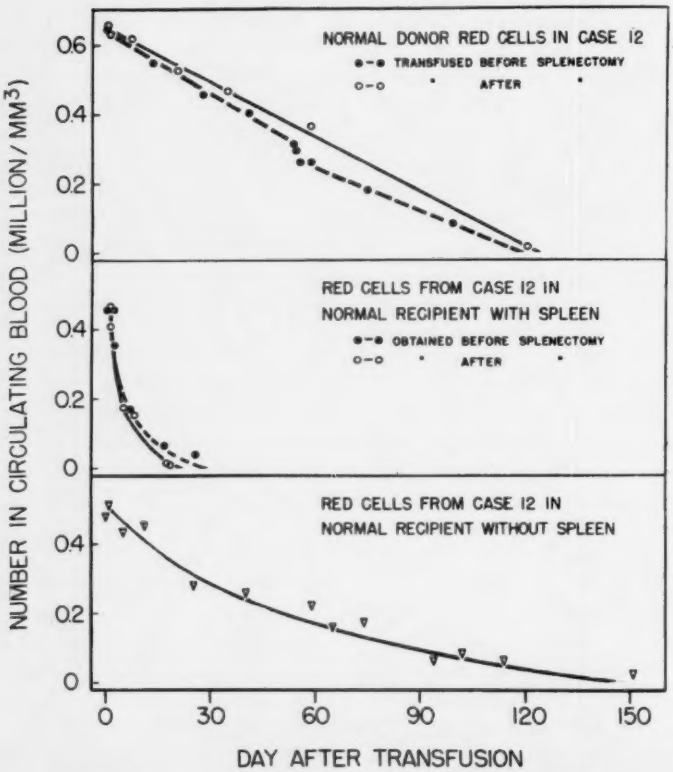


Fig. 4.—Post-transfusion survival of normal red cells transfused into Case 12 before and after splenectomy compared with the survival of red cells from Case 12 in normal recipients with and without a spleen. The survival of the normal donor's red cells was normal when transfused into Case 12 on two occasions—once before and once after splenectomy. A normal recipient with spleen intact was transfused on two occasions with red cells obtained from Case 12—once before splenectomy and, again, after splenectomy of the patient. In each instance the rate of destruction of the patient's red cells was approximately equal and markedly increased over that of the normal. However, when the recipient without a spleen was tranfused with red cells from Case 12 the survival was markedly prolonged and approached that of normal cells. (The spleen of the normal recipient had been removed nine months previously because of traumatic rupture.)

symmetric, it may be inferred that the variation in spheroidicity also is distributed in a symmetric manner.

These red cells, unmodified by the spleen, and therefore representing bone marrow production, appear to be malformed and permanently defective. As long as the spleen remains in the body their life span is short; once the spleen is removed, however, they are not destroyed at a significantly increased rate. Their survival is short when they are transfused into other persons with spleens, but in a recipient without a spleen they survive normally. Thus, the presence of the spleen is clearly associated with the increased destruction of red cells. In the subsequent discussion the osmotic fragility curve obtained after splenectomy, usually within a period of 17 days, is used as a standard in each case for comparison with presplenectomy samples.

### 3. Characteristics of Red Cells in the Peripheral Blood as Observed Before Splenectomy in Hereditary Spherocytosis

*Experimental Data.*—Samples of peripheral blood obtained before splenectomy were studied in the manner described in Section 2, and the differences between the properties

of red cells before and after removal of the spleen were analyzed in search of evidence indicating the nature of its hemolytic activity. Twenty-eight patients were observed while their spleens were intact. In 13 of these cases splenectomy was performed during the course of this investigation. Data from these studies are recorded in Tables 4, 6, and 7 and illustrated in Figures 2, 3, and 5 to 10.

Patients exhibiting pronounced anemia were in the minority. The hemoglobin concentration was reduced below 11 gm/100 ml. in only 12 cases, the mean red cell count being 3,200,000±540,000/cu. mm. In 11 cases the hemoglobin level was between 11.0 and 14.0 gm./100 ml. and the mean red cell count, 3,900,000±270,000, while in the remaining 5 the hemoglobin concentration exceeded 14.0 gm/100 ml., the mean red cell count being 4,800,000±190,000/cu. mm. However, with but one exception (Case 26) all these patients exhibited an abnormally high level of reticulocytes, icterus index, or both.

The osmotic fragility of red cells studied before splenectomy differed in several important respects from that observed after removal of the spleen. Individual case studies illustrating these differences are shown in Figures 5 to 10, mean values being presented in Figure 3 and detailed data, in Table 4. In the presence of the spleen the curve of osmotic fragility of peripheral blood was characteristically asymmetric and biphasic, reflecting a mixture of two or more distinct populations of cells. When compared

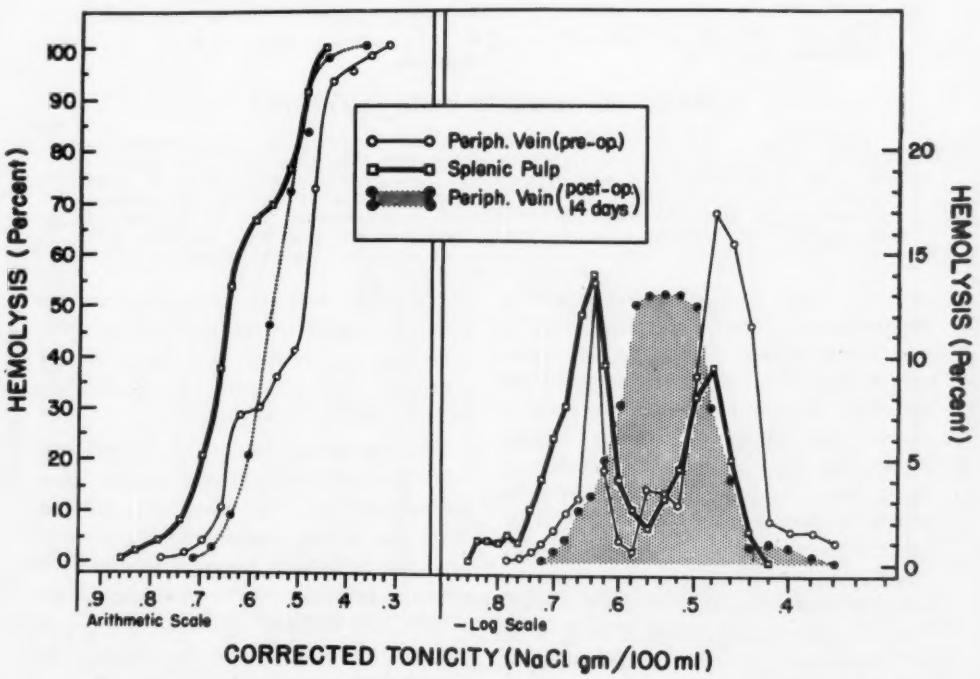


Fig. 5 (Case 9).—Osmotic fragility of red cells from the splenic pulp compared with that of the peripheral blood before and after splenectomy in a case of hereditary spherocytosis. The proportion of red cells with an increased osmotic fragility was approximately 30% in the peripheral blood and 70% in the splenic pulp, as estimated from the first phase of the biphasic summation curves. The maximum osmotic fragility of red cells from the splenic pulp exceeded significantly that observed in the peripheral blood. These observations suggest conditioning of red cells in the spleen and release of some to the peripheral blood. The asymmetry of the curve of osmotic fragility before splenectomy was extreme. See Figure 10 for a study of this patient postoperatively.

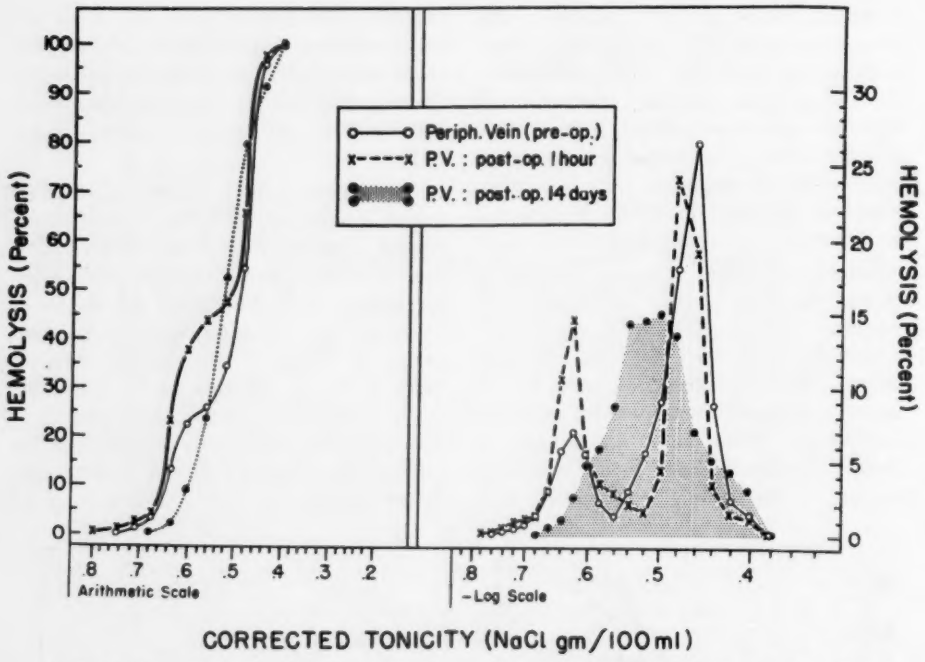


Fig. 6 (Case 1).—Osmotic fragility of red cells in venous samples, illustrating the immediate and late effects of splenectomy in hereditary spherocytosis. There was marked asymmetry of the curve before splenectomy, with approximately 25% of cells showing increased osmotic fragility. This was in contrast to the small per cent seen in Case 11 (Fig. 7). The asymmetry was greater one hour after splenectomy, with a rise to approximately 50% of abnormal cells. This indicates that abnormal cells were added to the peripheral blood from the spleen at the time of operation. Similar changes of lesser degree are shown for Case 6 in Figure 9.

with the curves obtained after splenectomy the preoperative curve regularly exhibited four distinguishing features, which varied only in degree from patient to patient: First, one of its components indicated the presence of red cells having an osmotic fragility greater than was ever observed after splenectomy. Second, a prominent peak appearing at tonicity levels above 0.6 gm/100 ml. indicated that cells with an elevated osmotic fragility were relatively more numerous before splenectomy than afterward. Thus (Figs. 5 and 6), approximately 25% to 30% of the cell population showed an asymmetric increase in osmotic fragility. Third, there was a remarkable paucity, or in some instances almost a complete absence, of red cells with an intermediate degree of osmotic fragility, i. e., those hemolyzing in the tonicity range between 0.50 and 0.60 gm/100 ml., as exemplified in Figures 5 to 7. A fourth point of difference was the frequent occurrence of another peak in the distribution curve, reflecting the presence of a large red cell population with a normal osmotic fragility, between 0.40 and 0.50 gm/100 ml.

The mechanical fragility of the red cells was significantly greater than normal before splenectomy and not materially different from that of the postsplenectomy samples, as shown in Table 7. When peripheral blood collected before splenectomy was incubated at 37 C, the red cells invariably showed an abnormal increase in osmotic fragility, the change being comparable to the effect of incubation on blood obtained after splenectomy, as shown in Tables 4 and 6 and in Figures 2 and 3. Incubation likewise produced a marked increase in the mechanical fragility of red cells present before as well as after splenectomy, as indicated in Table 7.

*Interpretation.*—Twenty-seven of twenty-eight patients with hereditary spherocytosis before splenectomy showed evidence of increased production and destruction of red cells, although five of these patients were not anemic and eleven had a minimal degree of anemia. When the osmotic fragility curves in individual cases before and after splenectomy were compared, significant differences were encountered, which may be explained by the following series of hypotheses. First, the large population of cells with a normal osmotic fragility present before splenectomy presumably were cells which had not been selectively removed but which had passed freely through the spleen and therefore represented a residue of relatively normal cells. Second, the absence of erythrocytes with an intermediate osmotic fragility suggests selection and sequestration by the spleen of types of susceptible cells which were later present in abundance after splenectomy. Third, the large population of cells with increased osmotic fragility, exceeding that of any cells observed after splenectomy, suggest that the spleen may have altered, without destroying, red cells which it had sequestered or delayed in transit and that many of these conditioned cells may subsequently have been released into the peripheral circulation. This hypothetical sequence of events, i. e., passage through the spleen, selection, sequestration, conditioning, and release of cells, was examined further by tests performed at the time of splenectomy on effluent blood from the spleen and on red cells extracted from the splenic pulp.

### 4. Characteristics of Red Cells from the Spleen in Hereditary Spherocytosis

The characteristics of the red cells from the splenic vein, the splenic pedicle, and the

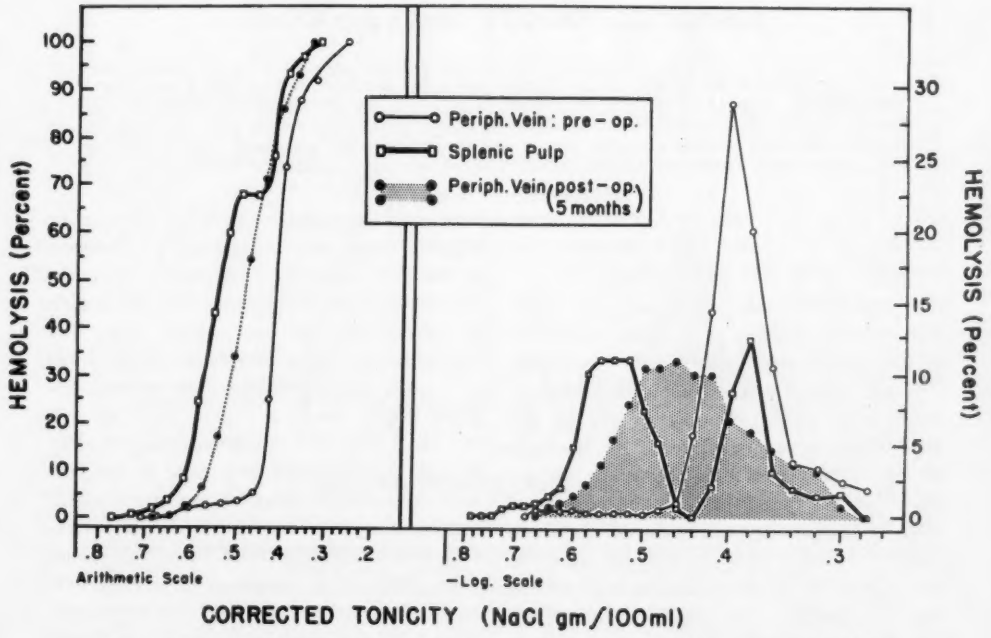


Fig. 7 (Case 11).—Osmotic fragility of the red cells in the splenic pulp compared with that of the peripheral blood before and after splenectomy in a case of hereditary spherocytosis. There was marked asymmetry of the curves before splenectomy. The proportion of red cells with increased osmotic fragility in the splenic pulp was approximately 70%, as was found also in Cases 4 and 9 (Figs. 8 and 5). However, the proportion of such cells in the peripheral blood was remarkably small, only 2%, suggesting a small release from the spleen. This is comparable to values obtained in Cases 4 and 6 (Figs. 8 and 9) and in contrast to the relatively large amounts released to the peripheral blood in Cases 1 and 9 (Figs. 6 and 5).

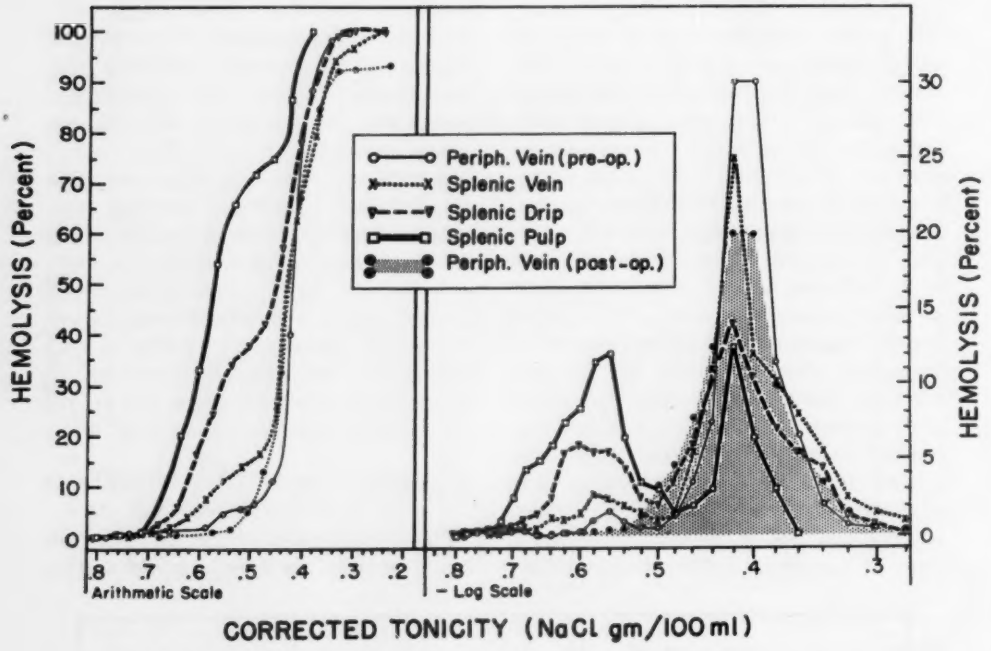


Fig. 8 (Case 4).—Osmotic fragility of red cells in different samples from the spleen compared with those in the peripheral blood before and after splenectomy in a case of hereditary spherocytosis. The curves show a progressive increase in the proportion of abnormal cells in samples from the peripheral vein, splenic vein, splenic pedicle, and splenic pulp. The osmotic fragility of spleen samples exceeded that of any sample from the peripheral blood before or after splenectomy. These observations suggest that red cells were conditioned in the spleen.

splenic pulp were compared with those in samples of peripheral blood obtained immediately before and after splenectomy.

*Experimental Data.*—Red cells with a normal osmotic fragility were most numerous in the peripheral blood, usually exceeding 70% of the entire population in these samples, as indicated most clearly by the summation curves in Figures 5 to 10. Cells of this type were least numerous in the spleen pulp, where they usually comprised 30% or less of the population, as shown in Figures 5 and 7 to 9. In samples from the splenic vein the number of cells with normal osmotic fragility was significantly greater than was encountered in the splenic pulp or in effluent samples from the pedicle of the excised spleen but was less than in the peripheral blood, as illustrated by the summation curves in Figure 8. Conversely, the number of abnormal cells was increasingly large, and the biphasic quality of the osmotic fragility curve was increasingly pronounced. in samples obtained, respectively, from the peripheral blood, the splenic vein, the pedicle of the spleen, and the splenic pulp. The concentration in the peripheral blood of red cells with abnormally elevated osmotic fragility ranged from a slight "trail" of 1% in Case 6 (Fig. 9*A*) to 30% in Case 9 (Fig. 5). As demonstrated in Figure 8, red cells with abnormally elevated osmotic fragility numbered approximately 5% in the peripheral blood, 18% in blood from the splenic vein, 40% in a sample from the pedicle, and 70% in the splenic pulp. Abnormal cells were more numerous in proportion to normal cells and the degree of abnormality was greater in the spleen pulp than in any other sample, especially when compared with the peripheral blood after splenectomy. Figure 3 shows the tonicity of mean hemolysis in

nine cases, demonstrating in composite form the striking difference between red cell populations in the spleen and those in the peripheral blood and between samples of peripheral blood collected before and those obtained after splenectomy.

The mechanical fragility of red cells from the splenic vein, pedicle, and pulp exhibited a progressive and marked increase, which correlated directly with the increase in proportion of cells with abnormal osmotic fragility, as shown in Table 4.

*Interpretation.*—There is presumptive evidence that red cells with normal osmotic fragility pass through the spleen unimpeded and undamaged, since they were encountered in large number in samples collected from the splenic vein and were relatively decreased in number in the splenic pulp. This is discussed further in Section 6. Concerning red cells with intermediate osmotic fragility, which were numerous after splenectomy, their absence from the peripheral blood before splenectomy suggests that they had been selectively and constantly removed, for it may be assumed that cells of this type were always being produced by the bone marrow. If the spleen were a perfect filter, pre-

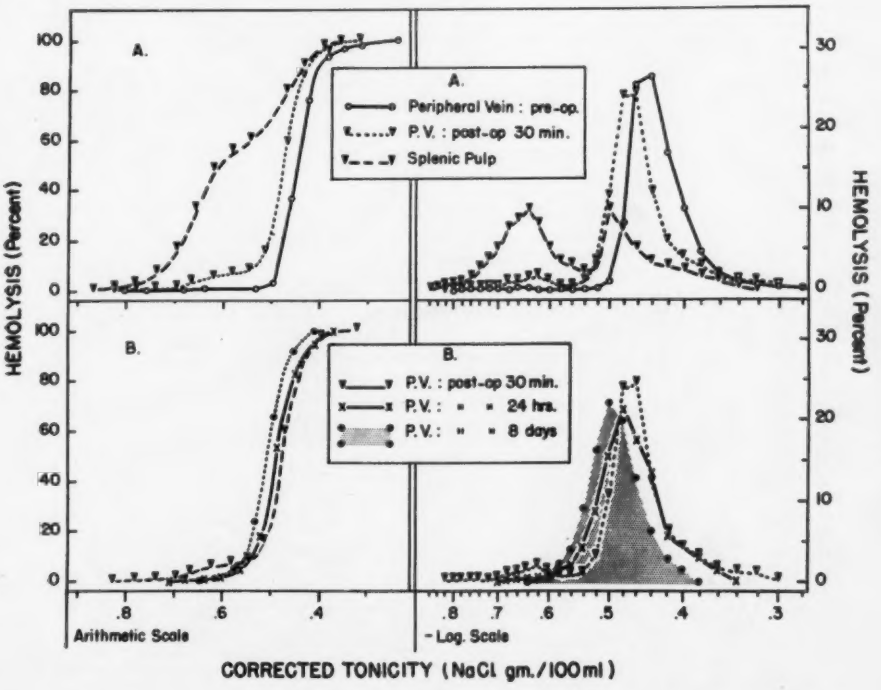


Fig. 9 (Case 6).—Osmotic fragility of red cells before and after splenectomy in a case of hereditary spherocytosis, demonstrating selective retention and destruction of abnormal cells by the spleen before its removal and dumping of these cells during splenectomy. *A*, just before operation an extremely small proportion of abnormal cells was present in the peripheral blood. Thirty minutes after splenectomy approximately 15% of cells of increased osmotic fragility were observed in the peripheral blood. (Note also in Figures 11 and 12 and Table 9 that this patient's red cell count increased from 2,000,000 to 2,670,000/cu. mm. immediately after splenectomy.) The spleen pulp showed approximately 70% of markedly abnormal red cells. This is evidence that cells of this type were selectively retained by the spleen before operation and then "dumped" back into the peripheral blood at the time of operation. *B*, within 24 hours after splenectomy these cells had completely disappeared, resulting in a symmetric distribution of the osmotic fragility. The rapidity with which they were eliminated suggests that this population had been conditioned by the spleen, their increased osmotic fragility reflecting their alteration and reduced capacity for survival.

sumably none of the abnormal cells would escape selection and removal by the spleen. As shown in Figures 7 to 9, this status is occasionally approached, the peripheral blood in these cases containing from 95% to 99% of cells with a normal osmotic fragility, in contrast to the splenic pulp, in which from 70% to 90% of the cells exhibited an abnormal increase in osmotic fragility.

Presumably spleens differ markedly as filters, however, for in some cases from 20% to 30% of the circulating erythrocytes were distinguished by having an abnormally increased osmotic fragility, as illustrated in Figures 5 and 6. In most cases, however, there was merely a "trail" in the osmotic fragility curve before splenectomy, indicating that only a relatively small proportion of the red cells avoided sequestration by the spleen or were released after having been conditioned by that organ.

Blood from the spleen pulp invariably contained red cells with an osmotic fragility greater than that observed in any other type of sample, including peripheral blood collected after splenectomy. It is unlikely that cells with these characteristics were produced as such by the bone marrow, since they were never observed after removal of the spleen. The hypothesis is advanced, therefore, that cells with an osmotic fragility greater than was observed after splenectomy had been conditioned in the spleen after selection and sequestration. It is evident, however, that such cells were capable of subsequent escape to the peripheral blood, because they were found in the splenic vein and also in the peripheral blood. Accordingly, there may

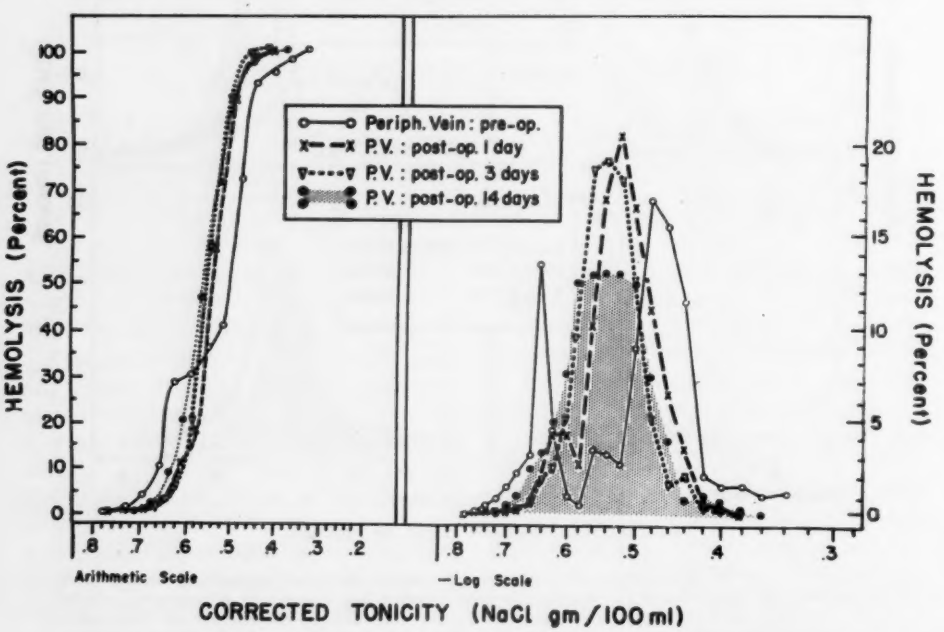


Fig. 10 (Case 9).—Osmotic fragility of red cells from the peripheral blood, showing the development of a symmetric curve after splenectomy in a case of hereditary spherocytosis. Red cells with a marked increase in osmotic fragility disappeared within a period of 24 hours after splenectomy. Within three days, red cells of intermediate osmotic fragility had reappeared in large enough number so that the curve was almost as symmetric as was observed 14 days postoperatively. Their previous absence suggests the selective removal of these cells by the spleen, as seen also in Cases 1, 4, 6, 9, and 11 (Figs. 5 to 9). Splenic retention of abnormal cells in this patient was relatively incomplete, however, as compared with Cases 6 and 11, whose peripheral blood before splenectomy contained almost none. See Figure 5 for a study of this patient at the time of splenectomy.

be release of some cells which have been conditioned by the spleen, especially during the manipulation of surgical removal.

Further evidence regarding the relative contributions of the spleen and bone marrow to the red cell defect in hereditary spherocytosis was sought by comparing the properties of red cells in the peripheral blood immediately postoperatively with those of cells obtained subsequently from the peripheral blood.

5. Characteristics of Red Cells in the Peripheral Blood Immediately Following Splenectomy

*Experimental Data.*—Blood samples were collected within a period of from 35 minutes to 8 hours after splenectomy in three patients, namely, Cases 1, 6, and 12. In each instance, as shown in Table 4 and in Figures 6 and 9, cells with markedly increased osmotic fragility were more numerous immediately after operation than before. In Case 1 (Fig. 6) their number increased from approximately 25% just before splenectomy to 45% one hour after splenectomy. In Case 6 (Fig. 9) the increase was from approximately 1% before splenectomy to 15% at 35 minutes after splenectomy. As shown in Table 9, the findings in Case 6 at 35 minutes after operation indicated that the concentration of patient's cells, as contrasted with donor cells, had increased from 2,000,000 to 2,670,000/cu. mm. The total number of cells in the peripheral blood increased approximately 15%, a figure which corresponded with the increase in fragile cells.

*Interpretation.*—Since the cells with strikingly abnormal osmotic fragility which appeared in the peripheral blood immediately

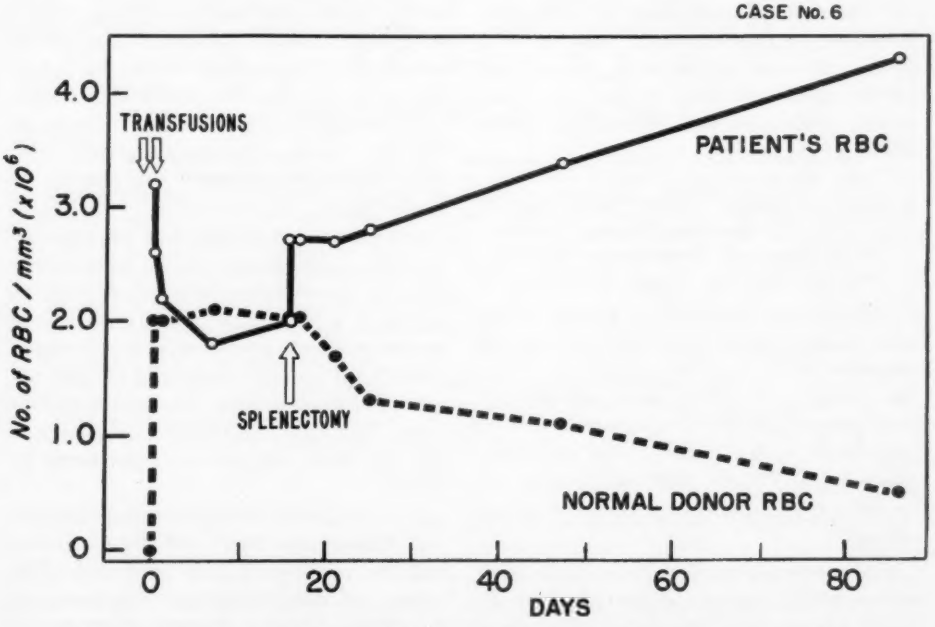


Fig. 11 (Case 6).—The influence of blood transfusions and splenectomy on the number of patient's red cells in the peripheral circulation in a case of hereditary spherocytosis. After transfusion of normal donor blood the number of patient's red cells decreased markedly, and the icterus index rose within 24 hours from 10 to 30 units, suggesting that increased hemolysis of the patient's cells occurred in response to transfusion. One-half hour after splenectomy, 17 days later, the patient's red cell count was found to have risen 33% above the preoperative level, which can only be explained by the assumption that these cells had been dumped from the spleen during the surgical manipulations. The donor cell count remained unchanged during the procedure (see Table 9). The change in osmotic fragility of the peripheral blood at this time is shown in Figure 9. The survival of the normal donor cells was normal, completely unaffected by the presence or absence of the spleen.

after operation exhibited the same characteristics as those extracted from the spleen pulp, it is reasonable to assume that their source was the spleen itself, whence they were expelled during operation. Distinguished by their osmotic behavior, these cells were readily detected, and their persistence in the peripheral blood could be estimated, as was done in Cases 6, 9, and 12. In each instance these highly abnormal cells were completely eliminated within 24 to 48 hours after splenectomy, as shown in Figures 9 and 10. This occurred at a time when the curve of osmotic fragility was rapidly becoming symmetric, owing to increasing numbers of red cells with an intermediate osmotic fragility. This occurred also at a time when the mechanical fragility of the red cell population was decreasing, as shown in Table 4, Cases 4, 5, 9 to 11, and 13. The rapid disappearance of these cells, coincident with the known decrease in blood destruction after splenectomy, is evidence for the rapid destruction of that population which, presumably, had already been conditioned by the spleen.

### 6. Characteristics of Normal Donor Cells in the Spleen and Peripheral Blood in Patients With Hereditary Spherocytosis and in Patients Without Hemolytic Anemia

The osmotic fragility of normal donor cells compared with those of the patient was measured in samples of peripheral venous blood and in the spleen pulp, and the survival of normal cells was determined after transfusion in five patients with hereditary spherocytosis. These observations were repeated in two patients without hemolytic anemia.

*Experimental Data.*—In two patients without hemolytic anemia (Cases 38 and 29, Table 5) who were transfused with normal donor blood, the proportion of patient's cells in the peripheral blood was identical to that observed in the splenic pulp, as shown in Table 8. The characteristics of the cells from the spleen in these cases are shown in Table 5 and Figure 1. However, in five patients with hereditary spherocytosis (Cases 4 to 6, 12, and 13) who had previously received normal donor blood the ratio of patient's cells to donor cells in the splenic pulp greatly exceeded that in the peripheral blood, ranging from 76% to 98% of the mixture, as shown in Table 8. The brief survival in a normal recipient of donor calls from a patient with hereditary spherocytosis (Case 12) has already been described in Section 2 and is illustrated in Figure 4. In Case 6 the survival of normal donor cells was normal over a period of 80 days, as shown in Table 9 and Figure 11. However, between the time of transfusion and splenectomy the number of patient's cells declined sharply, while the concentration of serum bilirubin increased. Associated with these changes was the disappearance from the peripheral blood of all but 1% of abnormally fragile cells, the pretransfusion level of these cells having been approximately 10%, as shown in Figure 9. Despite this evidence of active hemolysis of the patient's cells, the donor cells in the spleen of this patient at operation exhibited only a slight increase in osmotic fragility, whereas the patient's own cells were extremely abnormal, as shown in Figure 12.

In Cases 4 and 5 there was likewise observed a slight increase in the osmotic fragility of donor cells in the splenic pulp as compared with that of donor cells in the peripheral blood at the time of operation, whereas the osmotic fragility of the patent's own cells in the spleen was markedly elevated. The total survival of normal donor cells in these patients was approximately normal.

In Case 13 the destruction of donor cells was abnormally rapid and their osmotic fragility was significantly increased. This patient had received multiple transfusions in the course of earlier hospital treatment for bleeding peptic ulcer and subtotal gastrectomy. Although isoimmunization may have been responsible for the rapid destruction of donor cells in this instance, proof of incompatibility was not obtained and the mechanism of the increased osmotic fragility remains obscure.

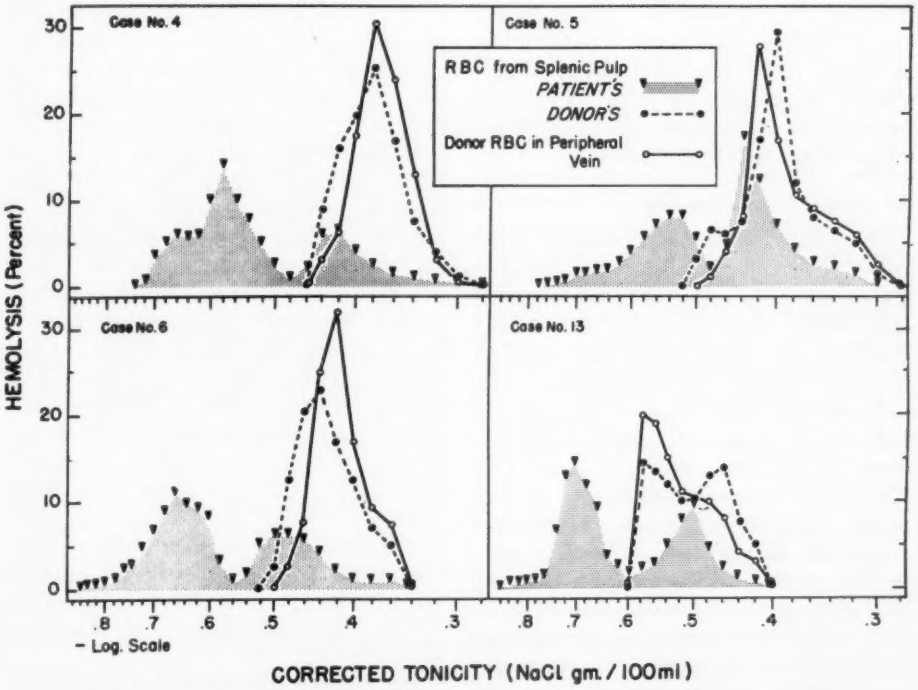


Fig. 12 (Cases 4, 5, 6, and 13).—Osmotic fragility of the patient's red cells compared with donor cells in venous samples from the spleen in four cases of hereditary spherocytosis. The osmotic fragility of the patient's red cells obtained from the splenic pulp is contrasted with that of the normal donor's red cells in the splenic pulp and with donor red cells in the peripheral blood immediately before splenectomy. Transfusions of normal donor blood were given to the patients at intervals of from 6 to 16 days before splenectomy. Observations were performed by the method of differential osmotic fragility, as described in the text and illustrated in Table 3. The patient's cells from the splenic pulp showed a marked increase in osmotic fragility, in contrast to the normal red cells in the same sample, which showed only a slight increase over that of the donor cells in the peripheral blood. Except in Case 13 the osmotic fragility of the donor cells was only slightly increased in the splenic pulp and their survival was normal for limited periods of observation. However, in Case 13 the osmotic fragility of the donor's cells in both the splenic pulp and peripheral blood was significantly increased and was associated with a rapid destruction of the donor's cells, as shown in Table 9. Although the mechanism of this rapid destruction is not known, this patient had received many transfusions previously because of bleeding from a peptic ulcer, and the possibility of isoimmunization, although not proved, cannot be excluded.

*Interpretation.*—Direct evidence has been obtained in vivo for selective removal of the patient's own red cells from the circulating blood and their sequestration in the spleen in cases of hereditary spherocytosis. These processes are associated with a marked increase in the osmotic fragility of the patient's red cells in the splenic pulp, normal donor cells in the same splenic pulp being only slightly affected. It is probable, therefore, that the spleen in hereditary spherocytosis functions normally, affecting normal red cells in a normal manner. No evidence indicating disproportionate selection or sequestration of red cells by the spleen was observed in patients without hemolytic anemia. The evidence further indicates that normal cells survive normally in hereditary spherocytosis. It also suggests that destruction of the patient's red cells may be increased following transfusion, as in Case 6, for example, all but 1% of cells with an increased osmotic fragility disappearing promptly from the peripheral blood. On the other hand, large

numbers of such cells were found in the spleen, whence they were "dumped" into the peripheral blood in the course of splenectomy.† This instance offers further evidence for selection, sequestration, conditioning, and release of red cells by the spleen.

## COMMENT

Historically, the disorder currently designated as hereditary spherocytosis, or congenital hemolytic jaundice, has been studied from the standpoint of the basic red cell defect, the role of the spleen, and the effect of splenectomy on red cell longevity.

The presence in these patients of spheroidal red cells with increased osmotic fragility was observed by Minkowski [1] and confirmed by Chauffard,[2] Naegeli,[35] Gänsslen,[36] Meulengracht,[37] and others.‡ Castle and Daland § established the relationship between the abnormal osmotic fragility of these cells and their geometric configuration, demonstrating that rupture of the nonelastic cell membrane occurs without stretching as the cell undergoes osmotic swelling and its form approaches that of a sphere. Accordingly, susceptibility to hemolysis in hypotonic solutions was interpreted by Castle and Daland as a function of a cell's capacity to swell before becoming spherical, this capacity being much greater in the case of a cell shaped like a biconcave disc than in one with a spheroidal body. This concept was studied by Haden [38] and by others,‖ and the osmotic fragility has become recognized as a quantitative function of red cell morphology as regards the degree of spheroidicity or biconcavity and the relative proportions of cells with various shapes.¶ The mechanical fragility of the red cell was observed by Shen, Castle, and Fleming [30] to be markedly and consistently increased in hereditary spherocytosis. Incubation of hereditary spherocytes at 37 C produces a striking increase in their osmotic fragility, as observed by Ham and Castle,# Young and co-workers,[45] and Selwyn and Dacie,[59] and in their mechanical fragility as well, as described by Shen and co-workers [30] and confirmed by Young and his co-workers.[45] Other observers * have noted that the hemoglobin content of the red cells in hereditary spherocytosis is disproportionately high in relation to their volume, as indicated by an increase in the mean corpuscular hemoglobin concentration. Thus, it has been suggested that the major red cell abnormality in this disease may consist of an increase in the amount of protein,[46] hence osmotic activity and therefore water, within the cell relative to the area of cell membrane, resulting in a spheroidal shape. Conversely, Prankerd, Altman, and Young [60] have reported evidence for inborn errors of erythrocyte metabolism which they believe could be related to the spheroidal shape of the red cell and certain other aspects of hereditary spherocytosis.

Early observations pertaining to the spleen in cases of hereditary spherocytosis indicated that removal of that organ consistently relieved the anemia and jaundice of the patient but failed to eliminate the abnormal characteristics of his red cells.† Barcroft,[63] Dacie,[64] Ovahovats,[66] and others ‡ studied the anatomic features of the spleen and its behavior with respect to red cells in this disorder. Dacie [64] demonstrated that complete removal of red cells by perfusion was difficult or impossible in the case of spleens removed from patients with hereditary spherocytosis and inferred that the cells must have been sequestered in this organ to an abnormal degree. He observed remarkably little evidence for erythrophagocytosis in the spleen. Working with normal cats, Ovahovats [66] demonstrated a significant increase in the osmotic fragility of red cells obtained from spleen pulp, compared with those contained in the peripheral blood. Spleens removed from patients with

† The splenic vein was open during the manipulations, and so contracture of the spleen occurred in most instances.

‡ References 38-46.

§ References 3 and 4.

‖ References 44 and 47.

¶ References 48 and 55.

# References 56-58.

* References 38, 46, and 47.

† References 25, 41, 42, 61, and 62.

‡ References 25 and 67-71.

idiopathic thrombocytopenia were perfused by Young and co-workers [25] with mixed suspensions of normal red cells and cells from patients with hereditary spherocytosis, with the finding that the patients' cells were selectively removed from the perfusing mixture and retained in the spleen. Observations such as these suggest that differential selection of erythrocytes by the spleen might occur on the basis of a geometric peculiarity, such as increased thickness of spheroidicity of the red cell. This is supported by the observations of Bjorkman,[68] who demonstrated that animal spleens selectively retain particles having a diameter ranging from $3\mu$ to $4\mu$.

Transfusion studies by Dacie and Mollison [20] and others § have demonstrated that normal donor red cells survive normally in patients with hereditary spherocytosis. Conversely, the rate of destruction of hereditary spherocytic red cells is abnormally rapid when they are transfused into normal subjects.‖ However, Schrumpf ¶ found, as confirmed here, that hereditary spherocytic red cells survived normally when transfused into a normal subject whose spleen had been removed.

Evidence that hemolysins occur in hereditary spherocytosis [71] has not been confirmed. The finding of a positive Coombs test for globulin on hereditary spherocytes has been reported occasionally # but has not been a consistent finding in studies reported here or elsewhere.* There is no reliable evidence, therefore, for the participation of any antigen-antibody reaction in the hemolytic mechanism of hereditary spherocytosis. Nor is there evidence for agglutinins that may be related to increased osmotic fragility.†

Concepts from previous work, cited above, relate red cell spheroidicity to the removal of red cells by the spleen. The mechanism by which this removal is accomplished has not been defined, however, and the significance of spheroidicity that persists following splenectomy in the presence of clinical cure is still not understood. However, the spheroidicity remains as an evidence of the inherited defect in the red cells.

The mechanism of abnormal blood destruction in hereditary spherocytosis has been investigated further in the observations reported here, by studying the functions of the spleen relative to the red cell in patients with hereditary spherocytosis, in patients with no hemolytic disorder, and in normal subjects. The data obtained in the course of this study support certain hypotheses, which are summarized in the following sequence:

### 1. Activity of the Spleen in Hereditary Spherocytosis as Related to Normal Transfused Red Cells

In confirmation of earlier reports,‡ it has been established that normal compatible donor erythrocytes survive in normal fashion § after injection into patients with hereditary spherocytosis, irrespective of the presence or absence of the spleen. Donor cells obtained from the splenic pulp of such patients, with one exception,‖ exhibited an osmotic fragility which exceeded only slightly that of donor cells in the peripheral blood and were comparable in magnitude to those observed in the spleens of patients without hemolytic disease. However, the patient's own cells in hereditary spherocytosis found at the same time in the spleen showed increases in osmotic fragility which far exceeded those of the normal donor cells or of the patient's cells in the peripheral blood. It appears, therefore, that the spleen in hereditary spherocytosis does not affect the properties of normal cells to an abnormal degree and that the survival of normal cells is not decreased by its activities. However

§ References 21-23.
‖ References 20-23.
¶ References 69 and 70.
# References 72 and 73.
* References 45 and 74.
† References 12 and 56-58.

‡ References 20-23.

§ In one instance (Case 13) normal donor cells were rapidly eliminated from the circulation of the recipient, and the osmotic fragility of these cells was significantly increased. See Figure 12 and Table 9.

‖ In one instance (Case 13) normal donor cells were rapidly eliminated from the circulation of the recipient, and the osmotic fragility of these cells was significantly increased. See Figure 12 and Table 9.

extensively the spleen may be involved in the hemolytic mechanism, the destruction of red cells in hereditary spherocytosis is accomplished by this organ through the exercise of its normal functions. Indeed, the removal of the spleen from a normal subject appears to permit normal survival of red cells from patients with hereditary spherocytosis, which otherwise does not occur.

### 2. Activity of the Spleen in Hereditary Spherocytosis as Reflected by Changes in the Peripheral Blood After Splenectomy

Within a period of 24 hours or less following splenectomy changes affecting the red cell population had usually become evident and were proceeding swiftly. The red cell count rose abruptly and progressed without interruption to normal levels, the rapidity of this response reflecting the massive productive capacity of the marrow and the intensity of erythropoietic activity in these patients before and immediately following splenectomy.[75]

After splenectomy the increased number of red cells was accompanied by qualitative changes in the red cell population which resulted in a striking revision of the pattern of cell distribution in the peripheral blood. The osmotic fragility curves, previously asymmetric and biphasic, rapidly became symmetric and monophasic. The trend toward symmetry was pronounced by the second postoperative day and was completed within a period of two weeks. Symmetry, once achieved, was retained as a permanent feature of cell distribution. This sequence of changes suggests that the bone marrow in hereditary spherocytosis regularly produces a red cell population in which spheroidal cells are distributed in accordance with a regular and symmetric pattern based on degree of spheroidicity, or on the degree to which individual cells deviate from a norm with respect to some property directly related to this morphologic characteristic. It is reasonable to assume that a symmetric population is produced before, as well as after, splenectomy. Granting this assumption, then, deviations from symmetry observed before splenectomy are explainable on the basis of selective elimination of one segment of the population, owing, for example, to preferential retention of abnormal cells in the spleen. As an alternate hypothesis, the preoperative asymmetry might also be explained by some peculiarity related to the osmotic fragility of certain cells that may be abnormally susceptible to "conditioning" by the spleen, which are then released into the peripheral blood.

However, from a comparison of preoperative and postoperative fragility curves in cases of hereditary spherocytosis it becomes evident that the asymmetric character of the peripheral blood before splenectomy is due largely to a disproportionate loss of cells hemolyzing at tonicity levels between 0.5 and 0.6 gm. NaCl/100 ml. Moreover, preoperative samples contained a highly variable number of cells showing extreme increases in osmotic fragility, i. e., cells that hemolyzed at tonicity levels higher than were observed at any time after the third or fourth postoperative day. Conversion of the osmotic fragility curves to symmetrical shape followed, first, the elimination of the highly abnormal cells from the peripheral blood within two or three days and, second, the reappearance of that larger group of cells with moderately increased osmotic fragility. The remarkable rapidity with which the latter type of cell reappeared indicates that cells of this variety had been produced in large numbers before splenectomy. Conversely, the disparity in their numbers before and after removal of the spleen reflects the precision and effectiveness with which the spleen selects and destroys certain red cells in hereditary spherocytosis.

It has been demonstrated that the spleen has a striking predilection for red cells hemolyzing at tonicity levels above, rather than below, 0.5 gm. NaCl/100 ml., this figure marking approximately the dividing line between cells that were selected and those escaping selection by the spleen. The constancy of this relationship validates the osmotic fragility test as a measure of the susceptibility of red cells to selective destruction by the spleen in hereditary spherocytosis

and also implies that the mechanism of selection is based on some morphologic feature of the erythrocyte that relates directly to spheroidicity. It is interesting to note that this value, 0.5 gm. NaCl/100 ml., has critical significance in another connection, representing the maximum point of initial hemolysis in normal osmotic fragility tests. This may define a normal characteristic of the red cell as produced by the marrow or, on the other hand, it may reflect the similarity of spleen function in normal persons and in patients with hereditary spherocytosis.

### 3. Activity of the Spleen in Hereditary Spherocytosis As Reflected by the Character of Red Cells from the Spleen

Differential selection and trapping of red cells by the spleen in hereditary spherocytosis was observed on cells contained in the splenic pulp of patients who had previously received transfusions of normal blood. As described previously by Emerson and coworkers,¶ the concentration of patient's cells relative to donor cells was substantially greater in samples from the spleen than in those from the peripheral blood. Moreover, in contrast to the donor cells, the osmotic fragility of the patient's cells was far more abnormal in the spleen than in the peripheral blood. Thus the splenic pulp invariably contained not only a higher proportion of abnormal cells but also cells with greater abnormalities in osmotic and mechanical fragility than were observed in the splenic effluent or in the peripheral blood at any time. It became apparent that certain of those cells which had been missing from the peripheral blood were located in the spleen, as shown by the distribution curves obtained in Cases 6 and 11. In these instances the vulnerable cells were reduced to 1% to 2% in the peripheral blood, as compared with 70% in the spleen.

Extreme increases in osmotic fragility affecting a significant proportion of the spleen population are regarded as evidence that conditioning, as well as trapping, of these cells had occurred in the spleen. Trapping alone could scarcely explain their presence, since there was no indication that cells with similar characteristics were ordinarily detectable in the peripheral blood of these patients before their spleens were removed. Indeed, only during the first few hours after operation, but never after the second postoperative day, were cells of this type encountered in substantial numbers in the peripheral blood, and then they were found there presumably as a result of manipulation of the spleen during the surgical procedure. The occurrence of these abnormal cells afforded an opportunity to observe and to estimate the survival of conditioned red cells no longer subject to the influence of the spleen. The rapidity of their disappearance would seem to support the view that these cells were, in fact, conditioned and quite incapable of survival, even in a favorable environment. As an alternate hypothesis, it is possible that these cells, although conditioned, were rapidly rehabilitated and that their osmotic fragility could have decreased in the absence of the spleen.

The transient postoperative increase in conditioned cells has additional significance, moreover, in relation to the role of the spleen in hereditary spherocytosis as a source of conditioned cells, as opposed to its role as a trap for abnormal cells. From this evidence, trapping may not be considered necessarily as irreversible and permanent, since it appears that red cells that have been subjected to some degree of sequestration and modification in the spleen nevertheless can and do escape. Added support for this premise is derived from observations made at the time of splenectomy on several kinds of splenic effluent blood, indicating increasing degrees of abnormality associated with red cells derived from the splenic vein, blood drained from the splenic pedicle, and blood contained in the splenic pulp, considered in that order. These circumstances can be explained on the assumption that the "trapping" of abnormal red cells is followed by their conditioning and eventual egress from the spleen in a form which is more abnormal than occurred before entry. "Trapping" and "sequestra-

¶References 76 and 77.

tion" are possibly misnomers, to the extent that they may imply permanent confinement or absolute stasis, in contrast to a delay in transit. It is theoretically possible that periods of sequestration in the spleen may become progressively more frequent or longer until these cells with increasing abnormality are eliminated completely. According to this theory, then, blood destruction is abnormally rapid in patients with hereditary spherocytosis because, in contrast to normal persons, substantial proportions of the red cells they produce are spheroidal, hence vulnerable to trapping by the spleen. Consequently, splenectomy reduces blood destruction to normal levels, not by halting the production of spherocytes but by permitting these cells to survive, since they are not trapped or conditioned. This conclusion may be inferred also from the fact that spherocytosis persists and the number of cells with increased osmotic fragility is increased rather than decreased in these cases after splenectomy, despite cure of the anemia. Proof of this hypothesis has been established by transfusion studies in Case 12, demonstrating that red cells from a patient with hereditary spherocytosis were destroyed with equal rapidity, before and after splenectomy, when injected into a normal person with a spleen, whereas their survival was normal in a recipient without a spleen.

Certain of these hypotheses have been confirmed by Weisman and co-workers [78] by a different approach. A patient with idiopathic thrombocytopenia, but without anemia, was transfused 45 hours before splenectomy with 250 ml. of red cells, identifiable by Ashby agglutination, from a splenectomized donor with hereditary spherocytosis. At the time of operation the peripheral blood contained 3% donor cells, as compared with 49% in the splenic pulp. The sequestered donor cells obtained from the spleen showed marked increases in osmotic fragility and spheroidicity relative to their pretransfusion characteristics, in contrast to the normal cells in the spleen pulp. This observation is interpreted as direct evidence in vivo for the selective sequestration and conditioning of spherocytic red cells in the spleen of a recipient whose own erythrocytes were normal. The source of the abnormal cells was a patient with hereditary spherocytosis whose spleen had been absent for more than a year and who was no longer anemic.

Inherent peculiarities of the red cell in this disease include spherodicity, osmotic hemolysis at high tonicity levels, an increased tendency to hemolyze when subjected to mechanical agitation, and increase in osmotic and mechanical fragility when incubated. It is logical to speculate that three of these attributes, if not all four, are interdependent, reflecting a common defect, viz., a paucity of cell membrane, as a result of which the cell contents are increased in volume relative to the maximum capacity of that cell. Spheroidicity, the morphologic expression of that abnormality, has been cited as a logical explanation for the selective sequestration of such cells within the spleen, although the precise method of sequestration is unknown. The mechanism responsible for the "conditioning" of red cells in the spleen is likewise unknown, although the marked similarity between its effects, as observed in vivo, and the changes produced by incubation in vitro suggest that these may be analogous situations, the determining factor in both instances being isolation of the red cells from the circulating plasma.

## SUMMARY

The spleen in hereditary spherocytosis functions normally in relation to normal erythrocytes, as indicated by the fact that the life span of normal donor cells is normal in these cases. Moreover, the donor cells that are present in the spleens of transfused patients exhibit little or no increase in osmotic fragility, in contrast with the marked abnormality of patient's cells in that organ.

On the basis of observations in patients whose spleens were removed it is apparent that the continuous production of abnormal red cells is a constant and permanent feature of hereditary spherocytosis. The defect, which persists despite cure of anemia, is manifested by an abnormal increase in the osmotic

and mechanical fragilities of the red cells. These fragilities are markedly and abnormally increased by incubation at 37 C. After splenectomy the distribution curve of osmotic fragility is monophasic and symmetric and remains abnormally increased. These data indicate that the degree of spheroidicity, i. e., of osmotic fragility, is abnormally increased but is distributed as a norm curve about a mean at which the greatest number of cells hemolyze. This pattern of symmetric distribution is comparable to that of normal red cells, but the span in hereditary spherocytosis exceeds the normal range. After splenectomy in patients with hereditary spherocytosis the cells, despite their abnormality appear to survive normally, judging from the absence of signs for hemolysis. Also, the red cells from a patient with hereditary spherocytosis survive normally when they are transfused into a normal person without a spleen.

In the presence of the spleen, red cell destruction is abnormally rapid in hereditary spherocytosis. Distribution curves of osmotic fragility, instead of being monophasic and symmetric, are asymmetric and biphasic in most cases, owing to (*a*) the absence, apparently by removal, of a large proportion of the cells showing hemolysis at tonicity levels above 0.5 gm. NaCl/100 ml. and (*b*) the presence of cells with abnormal osmotic fragility which is greater than occurred in the same patient observed three days or longer after splenectomy.

There is direct evidence in vivo that the spleen in hereditary spherocytosis sequesters the patient's own red cells, which are characterized by their abnormally increased osmotic fragility and, therefore, by increased spheroidicity. The mechanism for this selective removal is not established but may be related to the abnormal spheroidai shape of the red cells, as suggested by previous investigators. The spleen appears to condition red cells which it sequesters, as evidenced by alteration of the osmotic and mechanical fragilities. Some of these conditioned cells are released to the peripheral blood, since they are demonstrable in blood from the splenic vein and also in the peripheral blood immediately after the manipulations that occur during splenectomy. Although the mechanism is not known for the final destruction of the red cells, it is probably related to the increase in osmotic and mechanical fragilities.

It is inferred from these observations that the function of the spleen in hereditary spherocytosis may be normal and that the inherited defect is limited to the red cells. On this basis it may be presumed that the normal functions of the spleen include the selective sequestration of spheroidal red cells from the general circulation and the conditioning of such cells in a manner which renders them incapable of prolonged survival.

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# Relapsing Febrile Nodular Nonsuppurative Panniculitis

**Pfeifer-Weber-Christian Disease; Report of Three Cases**

NINA POPOFF, M.D.
and
M. C. WHEELOCK, M.D., Chicago

Relapsing febrile nodular nonsuppurative panniculitis is a syndrome of unknown etiology. It is characterized by a recurrent, nodular, nonsuppurative, inflammatory process in the subcutaneous fat. The outbreak of lesions is usually, but by no means necessarily, accompanied by a low-grade fever. The nodules occur most often on the legs, thighs, abdomen, and breasts. They vary from a few millimeters to several centimeters in diameter. Rarely, similar manifestations have been noticed in epicardial, peripancreatic, perirenal, periadrenal, and mesenteric adipose tissues. A fatal case of this type was reported by Mostofi and Engleman.[11] The disease usually affects women, the ratio of the incidence being 3 : 1. The condition manifests itself most frequently between the second and fourth decades. Three cases in Negroes have been reported in the literature.

No specific therapy is known. Antibiotics and sulfonamides, especially sulfapyridine, have been used in some instances. Cortisone and corticotropin (ACTH) do not provide a constant desirable response. The recent German literature contains a case of Weber-Christian disease where response was favorable after a combined chlortetracycline (Aureomycin)-Pyrifer* treatment (H. Stuhlert[17]).

The characteristic clinical and histopathologic features were first described by Pfeifer,[12] in 1891. He reported a case of focal atrophy in the subcutaneous fat. The second was reported by Gilchrist and Ketron,[7] in 1916. The descriptive name of relapsing nodular nonsuppurative panniculitis as a clinical entity was first given by Weber,† who reported the third case, in 1925. "Febrile" was added to the existing title of the syndrome by Christian,[5] in 1928.

During the past decade and especially in the recent two-to-three years, this distinct form of panniculitis has been recognized with increased frequency. The literature up to this time contains 86 cases.

## HISTOPATHOLOGY

The clinical importance of Weber-Christian's syndrome is obvious, since no true anatomic or histopathologic differentiation among the multiple types of lipogranulomas is possible.

The formation of lipogranuloma is characterized by focal necrosis in the subcutaneous fat. The histopathologic picture changes with the time of its development. The early phase is characterized by diminution of the amount of fat within the fat cells—"simple atrophy." The nuclei become poorly stained and pyknotic. This process is accompanied by an inflammatory infiltrate consisting, in the beginning, of polymorphonuclear leucocytes, which give way more and more to lymphocytes and plasma cells. Disruption of

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From the Department of Pathology, Passavant Memorial Hospital and Northwestern University Medical School.

* Nonspecific stimulus therapy with bacterial poisons.

† References 19 and 20.

cell membranes leads to liberation of fat, which becomes saponified and appears as fine droplets. Formation of fatty-acid crystals incites the increase of granulation tissue. A later phase is characterized by the presence of macrophages loaded with fat and large multinucleated cells which have a foamy cytoplasm. In some instances, these cells surround the fat globules, assuming a flattened or cuboidal form resembling gland-like structures.

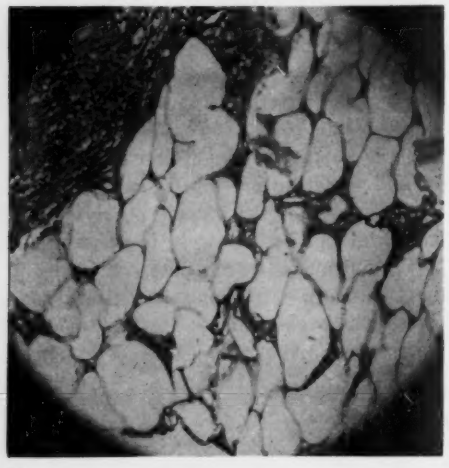
As the process proceeds, the focally atrophied fat becomes encapsulated or is replaced by a proliferation of connective tissue elements. The healing of large nodules results in shrinkage of the collagen fibers, which leads to a central area of dimpling.

## REPORT OF CASES

CASE 1.—A 28-year-old white woman, G., was in good health until the time of her last pregnancy, in April, 1953. At the end of the sixth month she began to have vaginal bleeding and was hospitalized for two weeks. Prior to this hemorrhage, she had a kidney infection, which had been successfully treated with sulfonamides.

At about seven and one-half months of the pregnancy the patient had another episode of vaginal bleeding. She was hospitalized again, and a third Caesarean section was performed under local anesthesia. Two previous pregnancies had been delivered by Caesarean sections, owing to cephalopelvic disproportion. Each time the surgical wound healed slowly. During the postpartum course the patient was kept on antibiotics.

Fig. 1 (Case 1, G.).—Photomicrograph of a later stage of relapsing febrile nodular nonsuppurative pannniculitis. There is interstitial inflammatory infiltrate among the fat cells, consisting mainly of lymphocytes, plasma cells, proliferated histiocytes, and several large multinucleated giant cells. Formalin fixation; hematoxylin and eosin; reduced from mag. × 100.



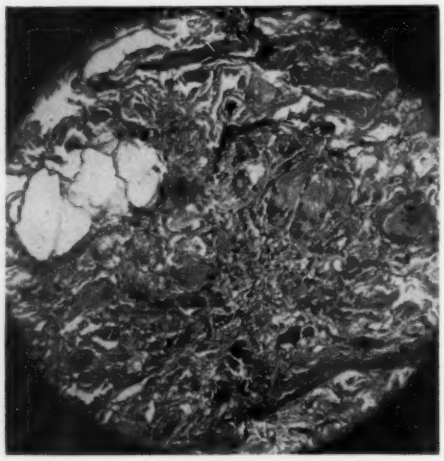


Fig. 2 (Case 1, G.).—High-power view of the inflammatory infiltrate, illustrating details of cells described for Figure 1; reduced from mag. × 170.

About the fourth postpartum week the first outbreak of tender erythematous lesions in the form of indurated subcutaneous swellings appeared on the anterolateral surface of the right lower leg. The nodules grew larger and produced elevation of the skin. After a period of several days they gradually subsided in size and changed to a purple color.

Meanwhile, successive groups of these nodules developed about this same area and followed the same course. Over a period of 10 to 11 months these swellings recurred at irregular intervals.

The patient had been treated by several physicians before her admission to this hospital. She received cortisone therapy; however, there was no effect on her lesions.

In December, 1954, the patient entered Passavant Memorial Hospital complaining of pain in the right leg and with symptoms of weakness and malaise. The temperature ranged from 99.4 to 100.1 F.

Physical examination revealed an asymmetric distribution of the above-described lesions on the anterolateral surface of the right leg. A few similar tender nodules had been present on the anterior surface of the left leg. These varied from 0.2 cm. to 1.5 cm. in diameter. The recovery from particularly large swellings resulted in dimpling and slight hyperpigmentation of the skin. Other physical

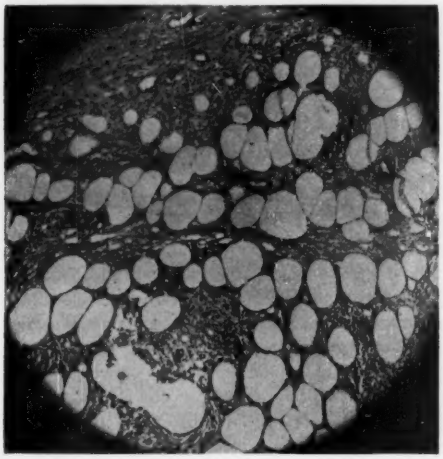
and laboratory findings were not contributory. Erythema nodosum had been considered clinically, and the patient was started on salicylates. Subsequently a few of the nodules were excised and submitted for diagnostic evaluation.

The histopathological findings were confined mainly to the subcutaneous fat. The overlying dermis revealed no changes other than mild telangiectasia. The interstitial inflammatory infiltrate among the fat cells consisted mainly of lymphocytes, plasma cells, proliferated histiocytes, and several large multinucleated giant cells, as is demonstrated in Figures 1 and 2. A moderate perivascular reaction and edema and thickening of the vessel wall were also noted. The diagnosis of chronic panniculitis compatible with that of Weber-Christian's syndrome, nonsuppurative relapsing febrile nodular panniculitis, was made by the department of pathology.

In the following two months the patient had a spontaneous remission and felt well until February, 1955, when she developed a severe upper respiratory infection which lasted about six weeks. At the end of March, 1955, she noticed a new outbreak of lesions on both lower extremities. The nodules were painful, erythematous, and this time more numerous. The largest lesion assumed the size of a "quarter."

CASE 2.—P., a 43-year-old married woman, suffered a subacute cholecystitis with cholelithiasis several years ago. She was successfully operated upon in March, 1949.

Fig. 3 (Case 2, P.).—Photomicrograph illustrating histopathological findings in a subcutaneous nodule removed five days after its outbreak. Cellular accumulation in the panniculus consists of lymphocytes, a considerable number of eosinophiles, histiocytes, and a few large multinucleated cells with foamy cytoplasm. Formalin fixation; hematoxylin and eosin; reduced from mag. × 85.



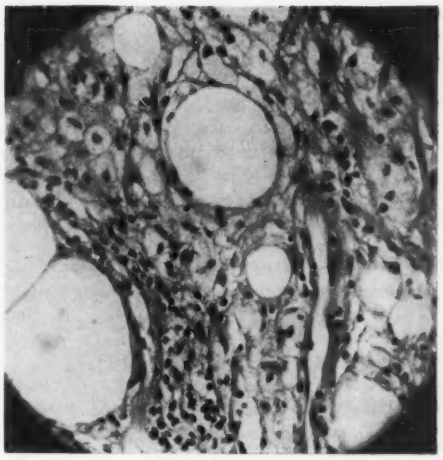


Fig. 4 (Case 2, P.).—High-power view of lesion seen in Figure 3, illustrating the details of cells; reduced from mag. × 390.

After leaving the hospital, the patient remained in satisfactory condition until August, 1953, when she started to complain of pain in the right upper quadrant. The liver was slightly enlarged and tender; however, all the liver tests performed at this time remained within normal limits and led to no diagnostic conclusion. About the same time she developed an excessive appetite for sweets and gained 10 lb. The patient complained of persistent fatigue and nervousness. A careful clinical checkup failed to disclose any suggestive factors for this condition except for the already known psychologic conflicts in the patient's marital life, which subsequently led to increasing symptoms of hypochondria. During a period of over a month's time, she received sodium bromide thrice daily. The patient states that she has been allergic to iodides in the past, which she received on one occasion several years previously as treatment for mild symptoms of hypothyroidism. She developed fine macular lesions extensively distributed over the body. The rash disappeared rapidly after immediate cessation of the therapy.

On Sept. 17, 1953, painful subcutaneous purplish-red discrete nodules appeared on both surfaces of the upper and lower extremities and the anterior chest. The swellings varied from 0.5 to 2 cm. in size. During the course of the following days, they increased in number, and the older lesions became blue. No liquefaction or suppuration of the nodules was noted. The patient's blood cell count remained within normal limits except for mild eosinophilia. The sedimentation rate became slightly elevated; however, it returned to normal after the lesions began to regress, within the second week after their first appearance. The outbreak of nodules had

been accompanied by a low-grade fever and mild symptoms of malaise. The patient received penicillin and erythromycin (Ilotycin) as treatment. The lesions subsided almost completely between the second and third weeks, leaving no residual dimpling effect of the skin. There have been no recurrences since that time.

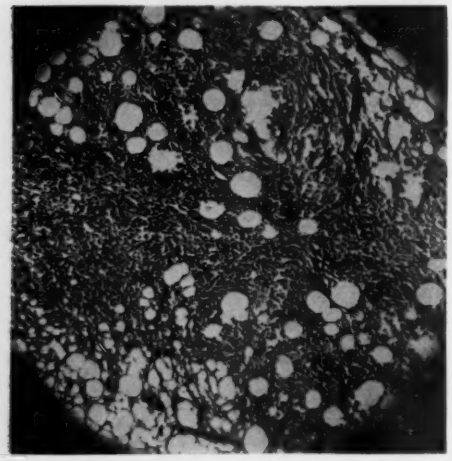
Two of the nodules taken from the calf of the right leg, which had been excised on the fifth day after their outbreak, were submitted for study. Microscopic findings revealed the epidermis to be well preserved. Several small blood vessels surrounded by lymphocytes were seen in the corium. Cellular accumulations in the panniculus consisted of lymphocytes, a few neutrophile leucocytes, and a considerable number of eosinophiles and histiocytes. A few large multinucleated cells with foamy cytoplasm were noted. The pathologic diagnosis was nonsuppurative nodular panniculitis, Weber-Christian disease (Figs. 3 and 4).

The patient is in good health at the time of this writing.

CASE 3.—B. M., a 48-year-old white woman, first noted painful indurated nodules in her right upper arm in the spring of 1952. At the time these appeared, she had a temperature of 100 F and had a tendency to tire easily.

The patient's family history was uneventful. She had the usual childhood diseases. At the age of 41 she was operated on for an acute appendicitis, and about four years later she had a cholecystectomy. Several weeks prior to the outbreak of the above-mentioned lesions, the patient recuperated from a severe upper respiratory infection. The nodules varied from 0.3 to 4 cm. in diameter. They gradually changed color from red to violet. Recovery from the especially large lesions resulted in centrally dimpled areas.

Fig. 5 (Case 3, B. M.).—Photomicrograph of relapsing febrile nodular nonsuppurative panniculitis. Acute stage. Interstitial inflammatory infiltrate consists of polymorphonuclear leucocytes, lymphocytes, monocytes, and a large number of eosinophiles. Formalin fixation; hematoxylin and eosin; reduced from mag. × 85.



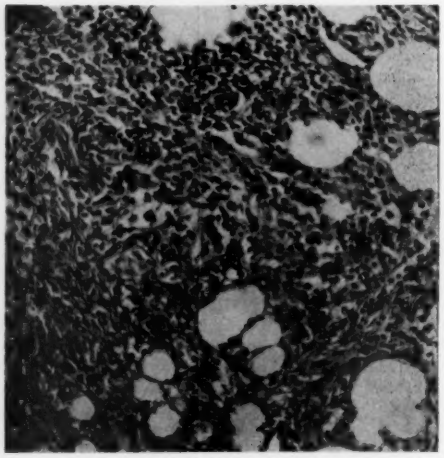


Fig. 6 (Case 3, B. M.).—High-power view of the lesion seen in Figure 5; reduced from mag. × 200.

A biopsy, with excision of several such nodules, had been done on July 26, 1952, and a diagnosis of Weber-Christian disease was made. The patient had also been studied at another hospital, where the same conclusion was reached. All the laboratory findings except for some hypochromia and relative polynucleosis remained within normal limits. The chest x-ray was negative, and the skin tests for histoplasmosis and coccidioidosis, as well as the tuberculin tests, were negative.

There were spontaneous remissions and relapses, which occurred at irregular intervals in the following two years. The distribution of nodules had been asymmetric, and they were seen on both upper and lower extremities as well as on the anterior chest.

Subsequent excisions with comparable conclusions had been made on Sept. 10, 1952; Nov. 2, 1953; April 12, 1954, and Aug. 19, 1954. The latest, in February, 1955, consisted of a small residual nodule which showed no activity.

Most of the encountered relapses had been preceeded by a mild upper respiratory infection. The patient received all forms of treatment, such as sulfonomides, antibiotics, and salicylates. She also had been treated for a short period with corticotropin (ACTH) and cortisone; however, there was no obvious improvement in the clinical picture. The lesions appeared in subsequent crops, which gradually underwent involution in two to three

weeks. During a later attack of the disease we were able to follow all of its morphological phases.

Figure 5 exhibits diffuse insinuation of subcutaneous fat by polymorphonuclear leucocytes, lymphocytes, monocytes, and proliferated histiocytes. Some areas show accumulations of a large number of eosinophiles.

Figure 6 is a higher magnification of the same picture.

Figure 7 represents histopathologic findings in a subcutaneous nodule removed about two weeks after its first appearance. Macrophages and large multinucleated cells with foamy cytoplasm dominate the picture. Disruption of cell membranes leads to liberation of fat, which appears in large globules.

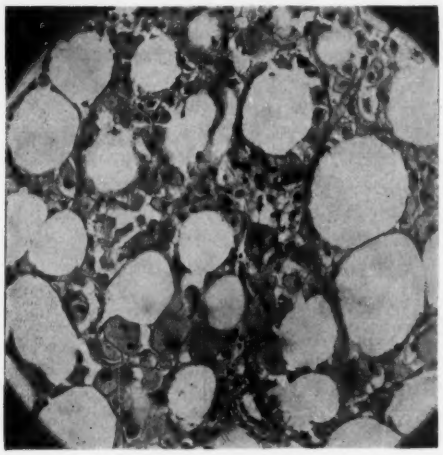


Fig. 7 (Case 3, B. M.).—Photomicrograph illustrating histopathologic findings in a subcutaneous nodule removed two weeks after its outbreak. The infiltrate consists mainly of macrophages and large multinucleated cells with foamy cytoplasm. In some areas these cells surround the fat globules, resembling gland-like structures. Hematoxylin and eosin; reduced from mag. × 200.

## COMMENT

Many unproved etiologic factors have been suggested by previous writers. The opinion of an inflammatory origin is generally accepted; however, the mechanism remains a question of debate. In 1916, a Russian pathologist, Abrikossoff,[1] described lipogranulomas in obese persons during the period of convalescence from infectious diseases. The nodules appeared predominantly in subcutaneous fat and also in retroperitoneal, omental, and mesenteric fat. In only two cases was it possible to demonstrate the association of the disease with a specific micro-organism. The first one, reported by Puente,[14] in Uruguay, was described as a lipogranuloma of Weber-Christian type of the breast caused by staphylococci. Sanford, Eubank, and Stenn[15] were able to culture a viridans Streptococcus simultaneously from a subcutaneous nodule and the nasopharynx in the same patient.

Hallahan and Klein[8] consider the association of Weber-Christian's syndrome with a sensitivity of some patients to iodides and bromides (halogens).

Trauma was observed to be the cause of recurrence of the nodules in a few cases (H. von Stuhlert[17] and Donald E. Taylor, W. M. Monroe, and W. Taliaferro[18]).

According to Bendel,[4] some noxious stimuli produce a metamorphosis in the subcutaneous fat which acts as a stimulus inciting a foreign-body response.

Brudno[2] reported a case of Weber-Christian's syndrome which occurred during a bout of recurrent rheumatic fever. He regards the syndrome as an allergic systemic reaction to nonspecific stimuli with focal manifestations. He believes that an antigen-antibody reaction is responsible for the disruption of the cell membranes and subsequent liberation of fat.

The variable response to different kinds of antibiotics does not permit one to accept the bacteriologic pathogenesis of the syndrome. At the same time, importance should be assigned to the fact that in most of the reviewed cases in the literature, as well as in our own cases, the syndrome arises in relation to another disease. In many patients outbreaks of nodules and, especially, recurrences follow an upper respiratory infection. In a case reported by London and Lev[10] the patient had had 10 relapses, each developing after an upper respiratory infection. The same is true for Case 1 and Case 3, described in this paper.

In Case 2 a supposition of hypersensitivity towards the halogen group can be accepted. The patient reacted twice with an outbreak of lesions; the second time the

manifestation resulted in typical lipogranulomas of Weber-Christian type.

A large number of eosinophiles found within the nonsuppurative lesions indicates an allergic disorder of an atopic type. It may be assumed that some patients have an inherited tendency to become sensitized by nonspecific stimuli, which can be bacterial, viral, or chemical in origin. The existing latent allergic state in such patients would be reactivated by an additional similar antigenic stimulus. For example, during the course of an upper respiratory infection which could produce an anamnestic allergic response, the subcutaneous fat may have a peculiar predilection for this manifestation.

The increasing frequency of the diagnosis of Weber-Christian syndrome must be referred to better knowledge and clinical interpretation of the disease.

SUMMARY

Three cases of Weber-Christian syndrome are added to the growing literature on this subject.

The histopathology of the lipogranulomas is discussed.

The previously described suggestive etiological factors are briefly summarized.

A further postulation is offered of a possible inherited tendency in some persons to become sensitized and to react later to additional similar antigenic stimuli.

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# Free Reducing and Hydrocortisone-like Steroids in Human Plasma

**Study on Healthy and Diseased Subjects**

SMITH FREEMAN, M.D., Ph.D.
J. X. WHEELER, B.S.
and
H. W. HOEGEMEIER, M.D., Chicago

One of the earliest attempts to estimate chemically the concentration of adrenal cortex steroids in blood was made by Hemphill and Reiss, who measured the total reducing ability of blood-lipid extracts by means of the Hagedorn-Jensen blood sugar method.[1] In 1948, Corcoran and Page[2] applied the procedure for the measurement of formaldehydegenic steroids to extracts of blood plasma. Recent chemical methods have provided a more specific means for the measurement of adrenocortical substances with a 17-hydroxy-ketol side-chain, such as occur in compounds E (cortisone), F (hydrocortisone), and S.

In 1952, Nelson and Samuels[3] reported a method for the direct quantitation of 17-hydroxy-adrenocortical steroids. According to their procedure, the blood extract was purified by solvent partition and chromatography on a synthetic magnesium silicate (Florisil) column. The 17-hydroxy blood corticosteroids were estimated by a micro-modification of the phenylhydrazine reaction as introduced by Porter and Silber,[4] which is stated[5] to be a specific test for 17,21-dihydroxy-20-ketosteroids. Similar results[6] on plasma were obtained by Sweat and Farrell,[7] who based quantitation on the fluorescent properties of compounds B (corticosterone) and F. The presence of an alpha-beta unsaturated ketone in ring A and a hydroxyl group at C-11 are regarded as necessary for the production of a high degree of fluorescence by adrenal cortex steroids.

Morris and Williams[8] combined partition chromatography and polarographic estimation of the keto-hydrazones of delta 4-3 ketosteroid groups as a basis for determining adrenocortical steroids. These authors separated compounds A, B, E, and F from the total blood "corticoid" extract.

Recently, Chen, Voegtli, and Freeman[10] described a method for the estimation of the total plasma reducing steroids based on the reduction of tetrazolium blue. This method yields values for plasma corticoids that are significantly higher than those obtained by the phenylhydrazine reaction. This difference is presumably due, at least in part, to the presence in the circulation of extractable steroids with a reducing side-chain that does not have an hydroxyl group on position 17.

The blood corticoid values reported by various investigators are summarized in Table 1.

It is apparent that the adrenal gland liberates several steroids into the circulation. Alterations in adrenal function that may occur in disease states could be reflected by changes in the absolute or relative amount of steroids liberated. The simultaneous determination of the plasma total extractable

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From the Veterans Administration Hospital, Hines, Ill., and the Department of Biochemistry, Northwestern University Medical School, Chicago.

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and "F-like" steroids should give some additional basis for judging adrenal cortex activity as compared with the study of either fraction separately.

The present report contains "corticoid" values obtained by the tetrazolium method for total extractable reducing steroids (tetrazolium-blue-reducing steroids [TBRS]) and values for hydrocortisone-like compounds (phenylhydrazine-reacting steroids [PRS], "F-Like") by the phenylhydrazine reaction. Studies have been carried out on healthy subjects of various ages and on patients with a variety of diseases. Observations on healthy subjects include the determination of the diurnal variation in plasma corticoid concentration and its constancy in the plasma of a given person at intervals for a year or longer.

TABLE 1.—*Published Methods for the Estimation of Plasma "Corticoids"*

| Author and Reference | Method | Range of Normal Values, γ/100 Ml. Plasma |
|---|---|---|
| Nelson & Samuels [8, 5] | Phenylhydrazine reaction | 4-10 |
| Bliss, Sandberg, Nelson, & Eik-Nes [6] | Phenylhydrazine reaction | 2-34 (Av. 13±6) |
| Bliss, Nelson & Samuels [17] | Phenylhydrazine reaction | 8-31 (Av. 19.2) |
| Morris & Williams [8] | Polarographic estimation of the ketohydrazones | 15.5-33.0 (total based on the sum of fractions A, E, F, & B) |
| Sweat & Farrell [7] | Sulfuric-acid-induced fluorescence | 6.0-10.0 |
| Chen, Voegtli, & Freeman [18] | Tetrazolium (BT) | 35.1±7.9 (male)<br>32.4±5.4 (female) |
| Present paper | Tetrazolium (BT) | 30.67±4.53 * |
| Present paper | Phenylhydrazine reaction | 20.51±3.62 * |

* Represents the average of 74 healthy adults in all age groups.

The separation of microgram quantities of steroids from other plasma constituents has been standardized to yield reproducible results. Values for total extractable reducing steroids estimated by the tetrazolium method in two independent laboratories have consistently shown agreement within the limits of error of the analytical procedure.

## GENERAL METHODS AND PROCEDURES

The methods for total free reducing plasma corticoids and F-like compounds referred to earlier were applied to the purified plasma extract. Ten to fifteen milliliters of heparinized plasma was used for the preparation of each extract, according to the procedure described by Chen and coworkers.* The chromatographic procedure for purification of the extract differed from the published method only in that each solvent used in the chromatographic separation was applied repeatedly to the paper strip rather than alternately. Either sequence was found to give the same results, and successive applications of the same solvent were more convenient. The area of the washed papergram to be cut out was determined by scanning each strip rapidly † under an ultraviolet light to localize the fluorescent steroids grouped in the vicinity of the "methanol front." The appropriate area in the paper strip (15-20 mm.) was cut out and transferred by means of clean forceps to a 2 ml. glass stoppered volumetric flask. (Corning No. 5640). Two milliliters of absolute ethanol was added to each tube, which was then stoppered tightly and allowed to extract overnight at room temperature. (Three hours has been found to be the minimum time required for complete extraction.) The contents of each tube was then mixed by inversion. A 1 ml. aliquot was removed for determination of total free reducing steroids, and the remainder was used for estimation of the "F-like" compounds by the Porter-Silber reaction, as described by Nelson and Samuels.[3] The use of microcells and a photomultiplier attachment to the Beckman DU spectrophotometer increases the sensitivity of the methods and permits reliable readings when there is a high per cent light transmission. Values obtained on small aliquots of concentrated ethanolic extract of pooled human plasma gave satisfactory agreement with the results obtained upon larger volumes of extract that were reduced to dryness at a low temperature and then redissolved in 0.2 ml. of ethanol, as required by the procedure for the Porter-Silber reaction. Unquestionably, the use of aliquots of the extract for two determinations reduces the accuracy of both methods. However, a comparison of values obtained on large and small aliquots of plasma extract indicates that the methods as employed

* References 9-11 and 15.

† These steroids are labile to ultraviolet light.

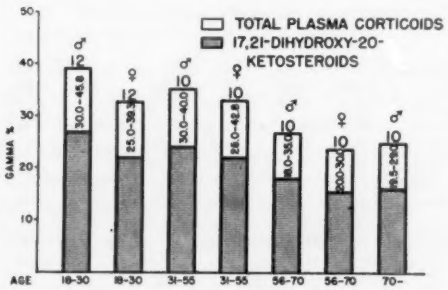



Fig. 1.—Effect of age on the concentration of total reducing and hydrocortisone-like steroids in the plasma of fasting healthy human subjects. Numbers at the head of each column refer to the number of subjects included in the group. Numbers inside the white portion of each column indicate the range of values for total reducing steroids obtained for the group.

were adequate for the purpose of this study. Each group of determinations was controlled by simultaneously carrying out the entire procedure on a blank paper strip and on a series of standards in saline put through the complete recovery procedure.

## RESULTS AND COMMENTS

Studies made on compounds F, B, E, Q, and S indicate that the recovery of the individual steroid added to plasma usually varied from 80% to 100% when 1γ to 3γ of steroid was added to 10 ml. of pooled human plasma. Recovery of a single pure steroid added to 45 ml. of saline and then carried through the entire analytical procedure was more complete (range, 94% to 100%). It is considered advisable to include aliquots of compound F added to saline with each group of analyses. These standard solutions, carried through the entire analytical procedure, serve as an additional check on the procedure and provide data from which is constructed the standard curve used in calculating the plasma corticoid values.

The normal values for the "F-like" steroids obtained in this study are higher than those reported by other investigators ‡ using the same reagent, although similar to some of the more recently reported values by Bliss, Nelson, and Samuels.[17] The principal differences in this procedure as compared with other studies employing the same reagent are in the extraction and chromatography procedures. The extraction wheel employed permits greater uniformity of extraction than with other methods. The use of silica gel columns, employed by others for separation of the steroids, has been associated with poor recoveries when applied to pure compounds in this laboratory. Attempts to demonstrate the inclusion of nonsteroid reducing substances in the portion of the paper chromatogram used for analysis have thus far led to negative results.

The fasting values for tetrazolium-blue-reducing steroids and phenylhydrazine-reacting steroids on a group of 74 healthy adults are shown in Figure 1. Average values for the entire group are 30.67γ per 100 cc. of TBRS and 20.51γ per 100 cc. of PRS. The average "F-like" compounds represent 66.87% of the total extractable reducing plasma "corticoids." The proportion of "F-like" compounds seems to be consistent for both sexes in all age groups.

‡ References 5-7.

TABLE 2.—*Diurnal Variations of Plasma Corticoids in Healthy Male Adults on Normal Daily Activity**

| | Fasting, 8 A. M. | | | 12 Noon | | | 5 P. M. | | | 10 P. M. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | TBRS† | PRS† | % "F"-Like | TBRS | PRS | % "F"-Like | TBRS | PRS | % "F"-Like | TBRS | PRS | % "F"-Like |
| 1 | 46.00 | 31.28 | 68.00 | 45.00 | 30.15 | 67.00 | 44.70 | 29.00 | 66.89 | 43.75 | 29.27 | 66.90 |
| 2 | 34.55 | 23.50 | 68.02 | 32.73 | 22.09 | 67.50 | 33.68 | 22.56 | 67.00 | 33.28 | 22.30 | 67.00 |
| 3 | 44.17 | 30.00 | 67.92 | 43.48 | 29.13 | 67.00 | 43.75 | 29.00 | 66.50 | 42.50 | 28.88 | 66.40 |
| 4 | 38.50 | 25.41 | 66.00 | 37.20 | 24.18 | 65.00 | 37.50 | 24.18 | 64.50 | 36.85 | 23.73 | 64.40 |
| 5 | 34.50 | 24.15 | 70.00 | 33.60 | 23.35 | 69.50 | 33.10 | 22.97 | 69.40 | 32.90 | 22.70 | 69.00 |
| 6 | 40.00 | 27.60 | 69.00 | 38.75 | 26.75 | 69.00 | 37.50 | 25.80 | 68.80 | 38.25 | 26.24 | 68.60 |
| 7 | 38.75 | 24.60 | 65.00 | 37.85 | 24.56 | 64.90 | 36.50 | 23.65 | 64.80 | 36.50 | 23.60 | 64.66 |
| 8 | 41.60 | 27.46 | 66.00 | 40.00 | 26.32 | 65.80 | 39.75 | 26.15 | 65.80 | 39.90 | 26.53 | 66.00 |
| Av. | 39.76 | 26.75 | 67.28 | 38.57 | 25.81 | 66.92 | 38.31 | 25.54 | 68.67 | 38.12 | 25.38 | 66.58 |

* Medical residents engaged in their daily routine on the wards.
† Tetrazolium-blue-reducing steroids (TBRS) and phenylhydrazine-reacting steroids (PRS) are expressed as micrograms of compound F per 100 ml. plasma.

TABLE 3.—*Variations in Plasma "Corticoids" of Healthy Adults over Several Months*

| | | 10/53 | | 1/54 | | 4/54 | | 7/54 | | 10/54 | | 1/55 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | Sex | TBRS* | PRS* | TBRS | PRS | TBRS | PRS | TBRS | PRS | TBRS | PRS | TBRS | PRS |
| 1 | F | 35.50 | 23.10 | 37.80 | 25.00 | 34.50 | 22.60 | 36.00 | 23.40 | 37.80 | 25.00 | 37.50 | 24.80 |
| 2 | M | 42.00 | 27.70 | 41.50 | 27.00 | 43.80 | 28.90 | 42.50 | 27.60 | 42.00 | 27.70 | 36.00 | 23.00 |
| 3 | M | 44.20 | 30.15 | 43.00 | 29.25 | 45.00 | 30.40 | 44.00 | 30.00 | 44.20 | 30.15 | ..... | ..... |
| 4 | M | 40.00 | 27.60 | 38.80 | 26.80 | 37.50 | 25.75 | 39.00 | 26.80 | 40.00 | 27.60 | ..... | ..... |
| 5 | M | 41.60 | 27.50 | 41.60 | 27.50 | 42.00 | 27.30 | 44.00 | 28.60 | 43.10 | 28.20 | ..... | ..... |
| 6 | F | 38.50 | 25.00 | 36.00 | 23.40 | 37.50 | 24.75 | 37.80 | 24.60 | 36.10 | 23.50 | 38.50 | 25.00 |

* Tetrazolium-blue-reducing steroids (TBRS) and phenylhydrazine-reacting steroids (PRS) are expressed as micrograms of Compound F (hydrocortisone) per 100 ml. plasma.

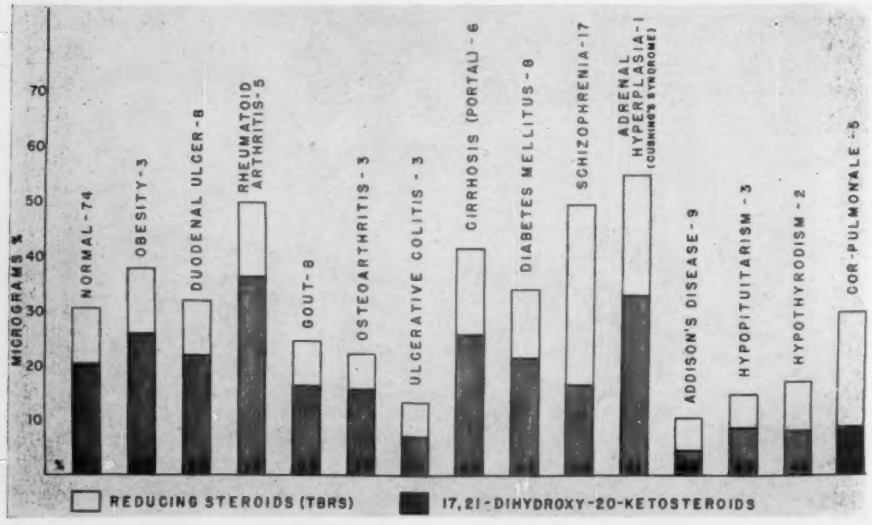
The male subjects of the youngest age group had on the average slightly higher "corticoid" values than females. This difference largely disappears in the older age groups. The TBRS and PRS values were proportionately lower for the older age groups of both sexes.

The diurnal variations of the plasma "corticoid" levels in eight male medical residents during their regular daily routine on the wards are presented in Table 2. Blood was drawn before each meal and before retiring. The steroid content was somewhat higher in the morning, although the values decreased only slightly throughout the day. The diurnal variation obtained is much less than that reported by Bliss, Sandberg, Nelson, and Eik-Nes.[6] The higher morning values are consistent with the data of Hoaglund, Romanoff, and Carlo,§ who found diurnal variations in urinary ketosteroids and reducing steroids.

The morning fasting blood corticoid levels of a number of healthy subjects were checked at intervals over a period of 12 to 15 months (Table 3), and a variation of approximately 3γ per 100 cc. was found. This variation is within the range of accuracy of the analytical procedures employed. The blood samples from the female subjects were not taken at any particular time with reference to the menstrual cycle.

§References 12 and 13.

Fig. 2.—Concentration of total reducing and hydrocortisone-like steroids in the fasting blood plasma in various diseases. The number following the name of each column indicates the number of subjects upon which the column average is based. The number at the base of each column indicates the average per cent of compound F for that column.



Results on 83 hospitalized patients with endocrine and nonendocrine diseases are summarized in Figure 2. The number of patients is too small to permit an adequate characterization of the several disease states included. However, an attempt was made to secure samples from patients with clinical findings characteristic of the disease and, insofar as was possible, uncomplicated by inanition. Debilitated and malnourished patients usually have a reduction both in the concentration of total plasma "corticoids" as well as in the per cent of "F-like" substances. In the majority of nonendocrine conditions, the proportion of total extractable ‖"corticoids" that are "F-like" ranges between 60% and 70%. Two exceptions to this statement are indicated by the results obtained on subjects with cor pulmonale and the groups of patients with acute manifestation of schizophrenia. Persons in these groups uniformly had a reduction in the per cent of "F-like" steroids. In the cor pulmonale group, the TBRS values were also lower than in the normal range of concentration, while in the group of untreated schizophrenic patients the reduced proportion of "F-like" substances was associated with normal or elevated values for the TBRS fraction.

The patients with untreated rheumatoid arthritis were remarkable in that their TBRS values were all in the upper limits of the normal range and associated with high absolute and relative values for "F-like" substances in their plasma. These values on patients with rheumatoid arthritis are considerably higher than those for "F-like" steroids reported by Perkoff and collaborators.[14] Five of nine untreated patients with clinical and laboratory findings typical of Addison's disease had TBRS values as low as 5γ per 100 cc. The patients with Addison's disease that had the higher TBRS values also had a significant plasma concentration of PRS compounds, although the per cent of "F-like" substances was reduced. Only one patient with Addison's disease failed to give significant plasma values by either method. The plasma TBRS values obtained in hypopituitary and hypothyroid patients were usually somewhat higher but occasionally overlapped values obtained on patients with Addison's disease. The PRS values for the patients with Addison's disease were consistently lower than for the hypothyroid and hypopituitary patients. The one patient with clinical and laboratory findings of Cushing's syndrome had adrenal glands that were removed and found to be approximately twice the normal weight. In this patient the values for both the TBRS and PRS fractions were higher than any encountered in healthy subjects.

As indicated in the introduction, there is still some difference of opinion regarding the amount and identity of the plasma corticoids. From the data presented here, it is evident that approximately two-thirds of the plasma reducing steroids as extracted and separated by the method described react with phenylhydrazine. According to Morris and Williams,[16] compounds A and B constitute approximately half or more of the plasma reducing steroids, with corticosterone (compound B) making up the greater proportion of the non-E and non-F fraction. Their results suggest that the difference between the TBRS and PRS values reported here largely represents compound B. Data to be presented in another paper demonstrate that the infusion of corticotropin into normal subjects is associated with an increase in both TBRS and PRS fractions in the same proportions as exist in normal fasting plasma. This finding supports the viewpoint that both fractions are largely adrenal in origin. If one accepts the above statement as correct, then the proportions of TBRS and PRS compounds in the circulation depend both upon the relative rates of elaboration by the adrenal gland and on uptake or metabolism by the tissues. Alterations in the proportions of these two fractions in several disease states may have either explanation. Further studies with corticotropin and with the administration of specific adrenal steroids may help to clarify the altered proportions

‖ According to Bongiovanni and co-workers,[18] part of the plasma corticoids are conjugated and not extractable by chloroform until hydrolyzed.

of the plasma corticoids observed in certain conditions.

Comparison of plasma and urinary steroid values in any detail is beyond the scope of this paper. However, it should be pointed out that some patients with normal urinary steroid values do not have a normal concentration or proportion of plasma corticoids and vice versa. Study of plasma corticoids may enable further differentiation of adrenal function not possible from estimation of urinary steroids alone.

SUMMARY

A comparison of total extractable reducing and "F-like" corticoids in the plasma was carried out on healthy and diseased human subjects. "F-like" substances were found to make up 60% to 70% of the total extractable plasma reducing corticoids of healthy subjects. The normal plasma corticoid concentration in a given healthy person was found to be quite constant and subjected to only a small diurnal variation. Both the absolute and relative amounts of tetrazolium-blue-reducing steroids and phenylhydrazine-reacting steroids were shown to vary from normal in certain diseases, while other diseases were associated with normal distribution of the plasma "corticoids" between the two fractions.

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# Capillary Microscopy in Rheumatic Fever

**The Capillary Patterns in Conjunctiva and Nailbed as Clinical Signs in Rheumatic Fever and Rheumatic Heart Disease**

**ELI DAVIS, M.D.**
**and**
**JACOB LANDAU, M.D., Jerusalem**

Capillary changes in rheumatic fever have been reported. There is evidence for increased capillary permeability (Wilson, 1940; Pratesi and Garofoli, 1952). Coburn (1933) found postmortem evidence that rheumatic fever was associated with diffuse hemorrhagic changes. Costero (1949) demonstrated at autopsy cerebral capillary hemorrhages in acute rheumatic fever. There is also a well-known connection between a tendency to nose-bleeds and rheumatism (Levine and co-workers, 1930). During capillary microscopy of the nailbed as a routine clinical examination and as a research tool (Davis, 1946; 1953) a particular capillary pattern was seen recurring in rheumatic fever and rheumatic heart disease. It was decided to study this pattern and compare its incidence in rheumatic fever and rheumatic heart disease with that in control patients. Likewise, the conjunctival capillaries were studied systematically by use of the slit-lamp (J. L.).

## METHODS

The technique of capillary microscopy at the nailbed and the normal pattern have been described repeatedly (Davis, 1946). For the nailbed examination we used simple apparatus—a standard medical microscope, a table fitting over the microscope platform as a hand support, and a powerful lamp for illumination. The table is fitted to a wooden base on which the microscope also rests. This arrangement minimizes movement of finger and microscope. Between the lamp and the objective is a globular flask containing green-tinted water to absorb heat and concentrate light. A drop of immersion oil is placed on the nailbed to be examined which rests in a special groove on the table over the microscope platform. At least two fingers, but usually three and often four fingers, of each hand are examined, often on several occasions.

The temporal bulbar conjunctiva from the limbus to the outer angle was examined by the slit lamp. Photographs were taken by fitting a 35 mm. camera to one of the slit-lamp binoculars in place of the eyepiece, while the visual observations were made through the second eyepiece. Illumination was done with a narrow beam of the slit lamp, and when the examined area was in focus the slit was opened to the full extent and the shutter was released (Dobree, 1954).

The normal pattern of the bulbar conjunctival vessels has been established (Duke-Elder, 1942; Berliner, 1949). A characteristic feature is the interlacing, anasmatosing network of the small vessels and capillaries.

This report is based on 100 patients with unequivocal acute rheumatic fever or rheumatic heart disease, who have been thoroughly examined and fully investigated and have been studied by combined examination of nailbed and conjunctival capillaries. Of our rheumatic patients 43 were male and 57 were female. Their ages ranged from 4 to 55 years. The rheumatic patients included 18 cases of acute rheumatic fever. There were 82 patients with chronic rheumatic heart disease ranging from the symptomless to the severest. Active and inactive cases were represented, and there were cases with the various kinds of valve lesions.

As a control group, the nailbeds and conjunctivae of 158 nonrheumatic patients were studied. The

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From Medical Outpatient Clinic "C" and the Ophthalmological Department of the Rothschild Hadassah University Hospital.

control patients (Table 2) included 30 cases of rheumatoid arthritis and 11 other patients with advanced collagen diseases. In addition, there were 13 patients with lupus erythematosus without systemic lesions and 13 patients with peripheral vascular diseases. There were five patients with Schönlein-Henoch purpura showing skin lesions and joint swellings, two cases of chronic purpura simplex, and four cases of chronic urticaria; three cases of chronic arthralgias of unknown etiology, and five cases of nephritis. There were 10 examples of various kinds of congenital heart disease and 42 patients with essential hypertension under the age of 50. As for chronic infectious diseases, there were six cases of chronic streptococcal tonsillitis and six cases of chronic bronchitis, three cases of chronic amebiasis, three cases of syphilis, and two cases of pulmonary tuberculosis.

Fig. 1*A*.—Photograph of rheumatic pattern of nailbed capillaries showing candelabras; × 20.

Fig. 1*C*.—Same as Fig. 1*A*; drawing.

Fig. 1*B*.—Same as Fig. 1*A*; reduced from mag. × 100.

Fig. 2.—Vasomotor instability pattern of capillaries; drawing.

## RESULTS

### 1. Pattern of the Nailbed Capillaries in Rheumatism

In rheumatism the capillaries appear to be at a greater distance than usual from one another and are often twice the average length. The arterial and venous capillary limbs are rather congested, particularly the venous, but the loops joining the limbs are regular. The subpapillary venous plexus is strikingly prominent. In addition, in a case classified as "positive" there were in each microscopic field capillaries forking from a common stem. This common stem may give rise to two or three or sometimes more capillaries, giving the appearance of a branched candlestick or candelabra (Figs. 1*A*, 1*B*, and 1*C*).This pattern was found in 31% of the rheumatic cases and in 4.5% of controls.

### 2. Pattern of Vasomotor Instability of Nailbed Capillaries

Although the picture to be described is by no means pathognomonic, it is sufficiently prevalent in our rheumatic fever and rheumatic cardiac patients to warrant special description. The pattern of vasomotor instability has been well described by Mueller (1922). The capillaries are two to three times the normal length and congested, particularly on the venous side. The special feature is that the loops joining the arterial and venous capillary limbs are markedly

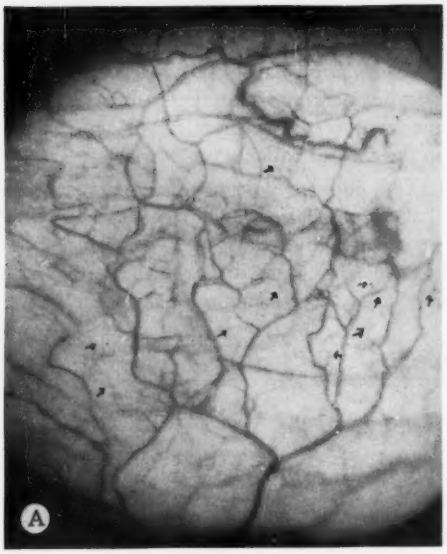


Fig. 3*A*.—Photograph of rheumatic pattern of conjunctival capillaries. Note arborization. Arrows point to end-vessels; reduced from mag. × 20.

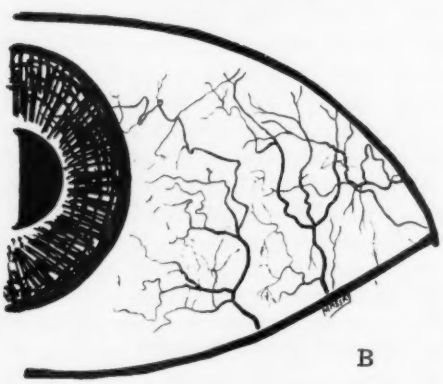


Fig. 3*B*.—Arborization and end-vessels of conjunctival capillaries; drawing.

irregular and strikingly convoluted in several capillaries in each field (Fig. 2). This pattern was seen in 7.7% of control patients and in 14% of rheumatic patients. The rather high incidence in rheumatic patients may prove to be significant. Apart from its occurrence in rheumatic fever and rheumatic heart disease, this picture was found particularly among patients suffering from rheumatoid arthritis.

### 3. Pattern of the Conjunctival Capillaries in Rheumatic Fever and Rheumatic Heart Disease

Seventy-nine per cent of our patients showed a special pattern of the conjunctival capillaries. The smaller vessels forked from a common stem and then subdivided repeatedly and more or less symmetrically, and many thinned abruptly. A number of the terminal subdivisions appeared isolated and, at our magnifications, without any apparent connection with neighboring vessels. This gave a picture which we call "arborization with end-vessels" (Figs. 3*A* and 3*B*). The pattern closely resembles the terminal branching of the walnut tree and the white poplar tree in winter. Tree-like branching alone was not considered pathological unless it was accompanied by end-vessels. This pattern was also seen in 7% of control patients, particularly in congenital heart disease and rheumatoid arthritis.

Detailed results are given in Tables 1 and 2. Analysis of our results did not reveal any correlation of the rheumatic pattern with sex or age or with acuteness, chronicity, or severity of the disease.

In 22 families in which there were 32 cases of rheumatic fever or rheumatic heart disease, 26 nonrheumatic parents or siblings of cases were examined. Among these 26—in whom there was no evidence of rheumatic fever or heart disease past or present—7

Table 1.—*Capillary Findings in Conjunctiva and Nailbed in Eighteen Patients with Acute Rheumatic Fever and Eighty-Two Patients with Rheumatic Heart Disease*

| Combined Findings * | Cases, No. | Separate Findings | Cases, No. |
|---|---|---|---|
| Conjunctiva +, nailbed + | 23 | Conjunctiva + | 79 |
| Conjunctiva +, nailbed — | 43 | Conjunctiva — | 21 |
| Conjunctiva +, nailbed VMI | 13 | Nailbed + | 81 |
| Conjunctiva —, nailbed + | 8 | Nailbed VMI | 14 |
| Conjunctiva —, nailbed VMI | 1 | Nailbed — | 55 |
| Conjunctiva —, nailbed — | 12 | | |

* +, rheumatic pattern; —, normal; VMI, vasomotor instability pattern.

had positive conjunctival signs and 5 had positive nailbed findings. This incidence is greater than the incidence in controls.

## Comment

A characteristic pattern of the vessels of the conjunctiva was seen in nearly 80% of patients with acute rheumatic fever or rheumatic heart disease in all stages, while in 31 of these 100 patients a characteristic capillary picture was found in the nailbed.

In the control group there was good representation of the collagen diseases, diseases involving the small blood vessels, and long-standing chronic infectious diseases. The incidence of the rheumatic capillary pattern in the control patients was low.

The findings in control patients suggest that the capillaries should be studied further in congenital heart disease and chronic streptococcal infection. It is worth noting that in two of the patients with congenital heart disease showing rheumatic patterns the presence of superimposed rheumatic infection could not be excluded.

Table 2.—*Capillary Findings in Control Patients*

| Disease | Cases, No. | Conjunctiva * | | Nailbed * | | |
|---|---|---|---|---|---|---|
| | | + | — | + | VMI | — |
| Rheumatoid arthritis | 30 | 3 | 27 | 2 | 4 | 24 |
| Acrosclerosis | 5 | 0 | 5 | 0 | 0 | 5 |
| Scleroderma | 4 | 0 | 4 | 0 | 1 | 3 |
| Dermatomyositis | 1 | 0 | 1 | 0 | 0 | 1 |
| Periarteritis nodosa | 1 | 0 | 1 | 0 | 0 | 1 |
| Lupus erythematosus (nondisseminated) | 13 | 1 | 12 | 1 | 1 | 11 |
| Raynaud's disease | 5 | 0 | 5 | 0 | 0 | 5 |
| Acrocyanosis | 4 | 0 | 4 | 0 | 0 | 4 |
| Thromboangiitis obliterans | 4 | 0 | 4 | 0 | 0 | 4 |
| Schonlein-Henoch purpura | 5 | 1 | 4 | 1 | 1 | 3 |
| Chronic purpura simplex | 2 | 0 | 2 | 0 | 0 | 2 |
| Chronic urticaria | 4 | 0 | 4 | 0 | 0 | 4 |
| Chronic arthralgias | 3 | 0 | 3 | 1 | 0 | 2 |
| Acute nephritis | 1 | 1 | 0 | 1 | 0 | 0 |
| Chronic nephritis | 4 | 0 | 4 | 0 | 0 | 4 |
| Congenital heart disease | 10 | 4 | 6 | 1 | 1 | 8 |
| Essential hypertension (patients under 50 yr. of age) | 42 | 0 | 42 | 0 | 2 | 40 |
| Chronic tonsillitis | 6 | 1 | 5 | 0 | 2 | 4 |
| Chronic infectious diseases, various (see text) | 14 | 0 | 14 | 0 | 0 | 14 |
| | 158 | 11 | 147 | 7 | 12 | 139 |

* +, rheumatic pattern present; —, rheumatic pattern absent; VMI, vasomotor instability pattern.

The fact that these patterns were seen at the first examination in patients with acute rheumatic fever (including patients aged 4 years) as well as in those with chronic and very long-standing disease suggests the hypothesis that the pattern may be present before the onset of the disease. The pattern may be a manifestation of a rheumatic diathesis rather than a sign of the disease itself. This view is fortified by the fact that these patterns are not infrequent among parents or siblings of patients, in whom there were no history and signs of the disease.

Why is the conjunctival pattern so much commoner than the nailbed pattern? The conjunctiva, a mucuous membrane with a rich capillary network, may reflect the state of the smaller vessels of the body more truly than the rather specialized loops of the nailbed.

As far as we have been able to ascertain, this is the first published report with combined observations of capillaries of the con-

junctiva and nailbed. The study of each area has helped the investigation, but the conjunctival capillaries are in general a more sensitive index of the abnormal morphology than those at the nailbed. On the other hand, in 8 of our 100 rheumatic patients the nailbed findings were positive when those of the conjunctiva were negative. A *Lancet* leading article (1954) stated that it may be assumed that the capillaries of the conjunctiva and nailbed fairly represent all the capillaries of the subcutaneous layer. In a number of patients not included in the above analysis it was impossible to interpret or see clearly the nailbed pattern, because of trauma, dirt, or manicure. In some of these patients a positive conjunctival result was obtained.

Analysis of the rheumatic patients with negative capillary findings both in conjunctiva and nailbed did not show any clinical differences between them and the patients with positive findings.

Pratesi and Garofoli (1952) have studied the nailbed capillaries (but not the conjunctival capillaries) in 18 cases of acute rheumatic fever and 11 cases of rheumatic heart disease. They studied capillary morphology by capillary microscopy and also investigated capillary permeability. They stated that the form of the capillaries was changed in all cases but that the permeability showed an increase in cases of acute rheumatic fever only. They stressed the importance of changes in the small vessels in the pathology of rheumatic disease, believing the changes to be partly constitutional and partly acquired. These changes, they believe, are secondary to the increase of capillary permeability.

We are in essential agreement with the conclusions of Pratesi and Garofoli, although we have used rather different criteria for defining our rheumatic pattern. While Pratesi and Garofoli found that the form of the capillaries was changed in all cases, in our 18 patients with acute rheumatic fever a positive nailbed result was obtained in 7 only, vasomotor instability pattern of the nailbed was seen in 3, and the nailbed capillaries were regarded as within normal limits in 8. The conjunctival pattern was positive in 13 of the 18. While Pratesi and Garofoli found more strongly positive capillary findings in acute rheumatic fever than in rheumatic heart disease, our capillary findings in both conditions were the same. While noting their view that the special capillary patterns are connected with increased capillary permeability, we suggest that these patterns are a manifestation of a rheumatic diathesis rather than of the disease.

Two practical applications of our findings are suggested: 1. Because of the very high incidence of the conjunctival sign in rheumatic disease and the appreciable incidence of the nailbed sign, in those cases where the diagnosis of rheumatic fever and rheumatic heart disease is presumptive but not conclusive the presence of these signs may weigh the balance in favor of the diagnosis. 2. The sign is frequent enough to warrant investigation of all patients previously unsuspected of having rheumatic heart disease who show these capillary patterns for its presence or for evidence of past rheumatic fever. It is of interest that in 6 of 10 patients who came to the eye department for purely local conditions in whom this pattern was found, hitherto unsuspected rheumatic mitral disease was revealed on further examination.

## Summary

The capillaries of the conjunctiva and the nailbed have been studied by both slit-lamp and capillary microscopy in 100 patients with acute rheumatic fever or rheumatic heart disease.

A characteristic capillary pattern was found in the conjunctiva of 79 patients and in the nailbed in 31 of these 100 patients—a considerably higher proportion than was found among control patients.

In the conjunctiva the special feature is the repeated subdivision of vessels (arborization) and the abrupt thinning of many of the terminal vessels, which often appeared to terminate as end-vessels, having no ap-

parent connection with adjacent vessels. This was in marked contrast with the usual picture of an interlacing latticework of the small conjunctival vessels.

In the nailbed the capillaries often branch from a common stem, giving the appearance of multibranched candlesticks (candelabras).

The possibility that the pattern may be present before the onset of rheumatism and may be a sign of a rheumatic diathesis is discussed.

The presence of these capillary signs may help diagnosis in borderline cases.

The finding of these signs may justify investigation of the symptomless patient for evidence of rheumatic heart disease.

Mr. Henry Tabor, Israel Scientific Council, gave advice and supplied the photographs.

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# Spiramycin

## Clinical and Laboratory Studies

DEAN G. HUDSON, M.D.
GEORGE M. YOSHIHARA, M.S.
and
WILLIAM M. M. KIRBY, M.D., Seattle

Spiramycin is a new antibiotic produced by a strain of Streptomyces isolated from soil samples in France.[1] The product was isolated by the French firm, Rohne-Poulenc Company. Sharp & Dohme, Division of Merck & Co., Inc., is exploring its clinical utility in the United States and supplied the spiramycin used in this study. The product is primarily a mixture of three chemical compounds, which can be separated but have not yet been completely differentiated. It is supplied in 0.5 gm. tablets, is hygroscopic, and has a bitter taste. Decomposition occurs rapidly at a pH lower than 4 or higher than 8.

This report concerns laboratory studies and a clinical trial with spiramycin in 29 adult patients with bacterial pneumonia.

### METHODS

*Sensitivity Tests.*—Tests to determine the concentrations of spiramycin necessary to inhibit the growth of streptococci and pneumococci were carried out in tryptose phosphate broth containing 3% human blood. For comparsion, sensitivity tests were performed simultaneously with penicillin and erythromycin. Amounts of 0.5 ml. of a $10^{-2}$ dilution of an overnight culture were added to equal amounts of broth containing various concentrations of the antibiotics. Four rows of five tubes each were employed. Tubes in the first row contained final concentrations of 50γ, 40γ, 30γ, 20γ, and 10γ or units per milliliter of the antibiotics. The other three rows contained a series of tenfold dilutions of these concentrations, with the smallest amount in the last tube being 0.01γ, or unit, per milliliter. The cultures were incubated overnight, and the lowest concentration of the antibiotic causing complete inhibition of growth by gross inspection was recorded. A similar method was used for staphylococci, except that blood was not added to the broth. In testing staphylococci, the final concentrations of the antibiotics were 50γ, 25γ, 10γ, 5γ, and 1γ per milliliter for spiramycin and erythromycin. For penicillin the values were the same, but the concentrations were expressed in units rather than in micrograms.

*Blood Levels After Oral Administration.*—All patients received an initial dose of 2 gm. of spiramycin, followed by 1 gm. every six hours. Specimens of blood were drawn two, four, and six hours after a dose was administered; in most cases specimens were not obtained until after several doses had been given. The blood was allowed to clot at room temperature, and the serum was separated and stored in the freezer until assays were performed. Spiramycin content of the serum was determined by bioassay, using macroscopic inhibition of a Group A Streptococcus in blood broth as the end-point.[2]

*Therapeutic Trial.*—Twenty-nine adult patients admitted to the King County Hospital with bacterial pneumonia were treated with spiramycin. The patients were predominantly in the older age groups; 10 were severe alcoholics; 25 patients were men, and 4 were women. All were acutely ill at the time of admission. Physical and x-ray signs of bacterial pneumonia were present in all cases. A direct smear of the sputum, a sputum culture, and a blood culture were done in each case prior to treatment in addition to the routine studies of blood and urine.

### RESULTS

*Sensitivity Tests.*—Results of sensitivity tests of recently isolated strains of streptococci and pneumococci to spiramycin, erythromycin, and penicillin are presented in Table 1. Pneumococci were found to be quite sensitive to spiramycin, although less so than to penicillin and erythromycin. Beta-hemolytic streptococci were in general less sensitive to spiramycin than pneumococci, and there was more variability from strain to

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From the Department of Medicine, University of Washington School of Medicine, and the King County Hospital.

TABLE 1.—*Results of Tube-Dilution Sensitivity Tests of Streptococci and Pneumococci*

| Organism | Source | Inhibiting Concentration | | |
|---|---|---|---|---|
| | | Spiramycin, γ/Ml. | Penicillin, Units/Ml. | Erythromycin, γ/Ml. |
| Beta-hemolytic streptococci | Throat or sputum culture | 0.5 | 0.01 | 0.03 |
| | | 1.0 | 0.03 | 0.04 |
| | | 0.2 | 0.03 | 0.04 |
| | | 0.5 | 0.02 | 0.03 |
| | | 0.2 | 0.03 | 0.02 |
| | | 0.4 | 0.10 | 0.03 |
| | | 1.0 | 0.03 | 0.03 |
| | | 0.3 | 0.03 | 0.02 |
| | | 1.0 | 0.03 | 0.03 |
| | | 2.0 | 0.03 | 0.03 |
| Average | | 0.7 | 0.034 | 0.03 |
| Beta-hemolytic streptococci | Cervix | 2.0 | 2.00 | 2.00 |
| | Retrocecal abscess | 0.2 | 0.10 | 0.03 |
| | Cervix | 1.0 | 0.02 | 0.02 |
| | Right hip ulcer | 0.5 | 0.02 | 0.05 |
| | Uterine discharge | 0.3 | 0.04 | 0.02 |
| | Urine | 2.0 | 5.00 | 0.20 |
| | Subhepatic space fluid | 2.0 | 5.00 | 2.00 |
| Average | | 1.1 | 1.74 | 0.61 |
| Nonhemolytic streptococci | Miscellaneous | 2 | 5 | 1 |
| | | 3 | 5 | 1 |
| | | 3 | 5 | 1 |
| | | 3 | 2 | 1 |
| | | 2 | 5 | 1 |
| | | 2 | 3 | 0.01 |
| | | 3 | 5 | 2 |
| Average | | 2.57 | 4.3 | 1.0 |
| Pneumococci | Sputum | 0.3 | 0.03 | 0.03 |
| | | 0.2 | 0.04 | 0.03 |
| | | 0.3 | 0.05 | 0.03 |
| | | 0.3 | 0.03 | 0.04 |
| | | 0.3 | 0.03 | 0.03 |
| | | 0.3 | 0.02 | 0.04 |
| | | 0.3 | 0.03 | 0.03 |
| | | 0.2 | 0.04 | 0.02 |
| | | 0.3 | 0.03 | 0.04 |
| | | 0.2 | 0.02 | 0.02 |
| | | 0.3 | 0.02 | 0.03 |
| Average | | 0.27 | 0.03 | 0.03 |

strain in the concentrations required to inhibit growth. Some of the beta-hemolytic streptococci isolated from sources other than throat and sputum cultures were undoubtedly enterococci. The nonhemolytic streptococci tested were less sensitive to spiramycin than to erythromycin, but in some instances the inhibitory concentrations of spiramycin were lower than those for penicillin.

Results of sensitivity tests of 117 strains of staphylococci freshly isolated from clinical specimens are presented in Table 2. Although they were less sensitive than to erythromycin, 93 strains were inhibited by 5γ per milliliter or less of spiramycin. Twenty-one strains required 10γ per milliliter, and three strains were resistant, growing in 50γ per milliliter. With erythromycin, 103 strains were inhibited by 1γ per milliliter; 1 required 10γ per milliliter, and 13 were highly resistant, probably as a result of therapy with this antibiotic. Of particular interest was the fact that there was little evidence of cross resistance between spiramycin and either penicillin or erythromycin. Of the 84 strains resistant to penicillin (growth in 50 units per milliliter) only 3 were resistant to spiramycin. Similarly, of the 13 strains highly resistant

TABLE 2.—*Sensitivity of 117 Strains of Staphylococci to Penicillin, Erythromycin, and Spiramycin*

| Strains Tested | Inhibiting Concentration | | |
|---|---|---|---|
| | Penicillin, Units/Ml. | Erythromycin, γ/Ml. | Spiramycin, γ/Ml. |
| 18 | >50 | < 1 | <10 |
| 55 | >50 | < 1 | < 5 |
| 3 | < 1 | < 1 | <10 |
| 22 | < 1 | < 1 | < 5 |
| 5 | < 1 | < 1 | < 1 |
| 2 | >50 | >50 | >50 |
| 1 | >50 | <10 | >50 |
| 8 | >50 | >50 | < 5 |
| 3 | < 5 | >50 | < 5 |
| 117 | | | |

to erythromycin, only 2 showed the same degree of resistance to spiramycin.

*Blood Levels.*—Serum concentration obtained on 28 occasions in 26 patients following oral administration of spiramycin are shown in Table 3. In general, the levels fell in a fairly narrow range, varying from 1γ to 6.7γ per milliliter. Up through the 11th dose the lowest value obtained was 1γ per milliliter; after the 11th dose, the lowest level was 2γ per milliliter, possibly indicating that some cumulative effect occurred after three days of therapy. There was a tendency for the highest levels to occur two hours after a dose, but in a number of instances the concentrations at four or six hours were higher than those recorded at two hours.

TABLE 3.—*Serum Concentrations of Spiramycin in Micrograms per Milliliter Following Oral Therapy*

| Patient | Dose Number | Spiramycin in Serum, γ/Ml. | | |
|---|---|---|---|---|
| | | After 2 Hr. | After 4 Hr. | After 6 Hr. |
| 1 | 2 | 3.0 | 1.5 | ...* |
| 2 | 2 | 2.5 | 3.3 | 2.5 |
| 3 | 3 | 2.5 | 4.0 | ... |
| 4 | 3 | 2.0 | 1.0 | 1.0 |
| 5 | 4 | 3.3 | 2.5 | 1.0 |
| 6 | 6 | 4.0 | 5.0 | 2.0 |
| 7 | 6 | 2.5 | 2.5 | 2.0 |
| 8 | 7 | 2.0 | 1.0 | 1.0 |
| 9 | 7 | 2.0 | 1.0 | 1.0 |
| 10 | 7 | 3.3 | 2.0 | 1.0 |
| 11 | 7 | 1.0 | 2.0 | 1.0 |
| 12 | 7 | 1.0 | 1.0 | 1.0 |
| 13 | 9 | 4.0 | 5.0 | 2.0 |
| 14 | 10 | 1.0 | 1.0 | 1.0 |
| 15 | 11 | 2.0 | 2.0 | 1.0 |
| 16 | 11 | 1.0 | 1.0 | 1.0 |
| 17 | 11 | 5.0 | 2.0 | 2.0 |
| 18 | 12 | 2.5 | ... | 2.5 |
| 19 | 14 | 2.0 | 3.3 | 2.0 |
| " | 38 | ... | ... | 2.0 |
| 20 | 15 | 4.0 | 2.0 | ... |
| 21 | 15 | 5.0 | 2.0 | 2.0 |
| 22 | 17 | 5.0 | ... | 3.3 |
| 23 | 18 | 2.5 | 2.0 | 2.5 |
| 24 | 18 | 2.0 | 2.0 | ... |
| 25 | 4 | ... | ... | 2.5 |
| 25 | 20 | ... | ... | 3.3 |
| 26 | 30 | 5.0 | 6.7 | 5.0 |

* Not done.

*Clinical Response in Patients with Bacterial Pneumonia.*—As noted in Table 4,

TABLE 4.—*Status on Admission of Patients with Bacterial Pneumonia*

| | Patients, No. |
|---|---|
| Age, yr. | |
| Under 50 | 5 |
| 50-59 | 6 |
| 60-69 | 8 |
| 70-79 | 7 |
| 80-89 | 3 |
| Total | 29 |
| Temperature | |
| Over 101 F | 22 |
| Under 101 F | 7 |
| Total | 29 |
| Leucocyte count | |
| Over 10,000 | 18 |
| Under 10,000 | 11 |
| Total | 29 |
| Sputum culture | |
| Usual flora | 11 |
| Pneumococcus | 17 |
| K. pneumoniae | 1 |
| Total | 29 |
| Blood culture positive for pneumococci | 2 |
| Concomitant diseases | |
| Alcoholism | 10 |
| Arteriosclerotic heart disease | 4 |
| Congestive heart failure | 2 |
| Pulmonary emphysema | 1 |
| Pneumoconiosis | 1 |
| Parkinsonism | 1 |
| Total | 19 |

the patients were mostly in the older age groups and were, in general, in rather poor condition. However, the clinical response in the patients with relatively uncomplicated pneumonias treated with spiramycin appeared to be comparable to results previously obtained with erythromycin and penicillin at this hospital.[3] In 26 of the 29 patients marked symptomatic improvement occurred during

the first two or three days and the temperature had returned to normal within five days of the onset of therapy. Included in this group was one of the two patients whose blood cultures were positive for pneumococci. This patient, an 87-year-old-white man with right lower lobe pneumonia, showed a prompt response and was afebrile within five days.

In two cases the course was more complicated. One patient, a 73-year-old white man with consolidation of the right upper and middle lobes, whose blood culture was positive for pneumococci, showed progressive improvement but required 10 days to become afebrile. The other patient was a 36-year-old Indian man with consolidation of the right middle lobe in whom improvement was slow. Penicillin and chloramphenicol were given after he had received spiramycin for five days. The temperature continued to decline slowly and was normal after 11 days. A delayed response similar to that occurring in these two patients has been observed on many occasions in this hospital with penicillin and other antibiotics, and the reason for it is not known. None of the patients in this series had pleural effusion or empyema.

There was one death, occurring in a 40-year-old Negro man with a fulminating left upper lobe and right middle lobe pneumonia, in whom there was no response to therapy with spiramycin. The initial sputum culture showed mixed flora, but subsequent cultures yielded a heavy growth of Klebsiella pneumoniae. Streptomycin was administered in large doses, but the patient died 72 hours after admission.

The principal untoward effect of spiramycin was a bitter taste, which was noted by a number of the patients when they did not swallow the tablets promptly. Two patients noted diarrhea on the second day of spiramycin therapy, but in both instances the diarrhea subsided within a few days even though the drug was continued.

## COMMENT

The present studies indicate that spiramycin is (1) quite effective in vitro against the commonly encountered Gram-positive bacteria, (2) well tolerated and well absorbed when given orally, and (3) clinically effective in the treatment of bacterial pneumonia.

Of particular interest was the fact that, with staphylococci, there was little if any cross resistance between spiramycin, erythromycin, and penicillin. Most of the penicillin- and erythromycin-resistant strains were sensitive to spiramycin, suggesting that this antibiotic may be a useful agent in the treatment of infections caused by antibiotic-resistant staphylococci. With spiramycin, however, the relationship between inhibitory concentrations in vitro and blood levels obtained in the patient is less favorable than with erythromycin and penicillin. A few staphylococci were inhibited in vitro by 1γ per milliliter of spiramycin, but the great majority required 5γ or 10γ per milliliter. When spiramycin was administered in doses of 4 gm. daily, blood levels of spiramycin varied from 1γ to 7γ per milliliter. It is apparent, therefore, that concentrations in the patient's serum may often be lower than those required to inhibit staphylococci in the test tube. Erythromycin, when given in doses only half as large (2 gm. daily), produces blood levels roughly the same as those obtained with spiramycin. Sensitive staphylococci are usually inhibited in vitro by 1γ per milliliter or less of erythromycin. No patients with staphylococcal infections were treated in the present study. The serum concentrations of spiramycin which are readily attained in the patient are at least 10 times as great as the concentrations required to inhibit pneumococci in vitro. The favorable clinical results obtained in patients with bacterial pneumonia are therefore not surprising.

## SUMMARY AND CONCLUSIONS

The results of laboratory and clinical studies of a new antibiotic, spiramycin, are presented. Pneumococci, streptococci, and staphylococci were inhibited by low concentrations of the antibiotic in vitro, but they were, in general, less sensitive than to penicillin or erythromycin. Of particular interest

was the fact that most strains of staphylococci resistant to penicillin or erythromycin were sensitive to spiramycin. As compared with erythromycin, however, the usefulness of spiramycin in the treatment of staphylococcal infections may be limited by the fact that staphylococci are less sensitive in vitro to spiramycin, and larger amounts are required to produce equivalent blood levels.

Spiramycin was well absorbed when administered orally in doses of 1 gm. every six hours, giving serum concentrations ranging from 1γ to 7γ per milliliter. The antibiotic was well tolerated. Some patients complained of a bitter taste, and two patients had diarrhea which was possibly caused by the antibiotic.

Favorable therapeutic results were obtained in a group of 29 patients with bacterial pneumonia. Twenty-six patients were afebrile within five days and made an uneventful recovery. Two patients ran a more prolonged febrile course, and one patient with Friedlander's pneumonia failed to respond.

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# Carbohydrate Metabolism

## I. A Study of Changes in Serum Inorganic Phosphorus During Glucose-Tolerance Test in Normals, Diabetics, and Prediabetic Women

MORTON D. KRITZER, M.D.
NORMAN SHRIFTER, M.D., M.S.
and
JAMES A. DEMETRIOU, Ph.D., Los Angeles

For some time it has been known that phosphate ion is involved in the intermediary metabolism of glucose.* The phosphorylation of glucose in transport to the liver and the subsequent changes in the metabolism of carbohydrate (as in the Embden-Meyerhof scheme), to mention only two, involve the participation of phosphorus radicals as necessary groupings. The subject is intriguing and has stimulated numerous investigators to perform both in vivo and in vitro experiments on the changes of phosphorus in metabolic processes. In the recent past, observations have been published linking changes in the serum inorganic phosphorus to the presence or absence of diabetes mellitus.[3] Thus, during the course of an intravenous glucose-tolerance test considerable drop in serum phosphate level was noted in the normal but a lesser fall in the diabetic. These values are considered important by those authors who emphasize that this change in phosphorus may be directly affected by the peripheral utilization of glucose. Furthermore, this test is advanced as a means of differentiating diabetes mellitus from impaired utilization of glucose by a defective liver.[3]

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From the Departments of Medicine and Pharmacology, University of Southern California School of Medicine, and the Diabetic Clinic, Los Angeles County General Hospital.

* References 1 and 2.

Experiments with radioactive phosphorus involving phosphate turnover in the intact rat yield conflicting results regarding differences between normal and diabetic animals.[4] The disparity in results of the two aforementioned sources may arise from the difference in experimental animals, i. e., man vs. intact rat, and the possibility that $P^{32}$ turnover may not reflect the same metabolic changes as the serum inorganic phosphorus fraction. Nevetheless, although many factors participate in the maintenance of a stable phosphorus level, carbohydrate metabolism is admittedly one of the most important.

It was planned to obtain serum inorganic phosphorus levels simultaneously with blood sugar values following glucose administration. Any observations would probably be of significance in adding another link to the rapidly growing chain of evidence in the medical literature concerning the relationship of phosphorus to carbohydrate. Previous work could be repeated and results, both corroborative and conflicting, presented.

A unique group of subjects was available for study. This consisted of a number of women, a significant percentage of whom will ultimately develop diabetes mellitus. These young women had each been delivered of a large baby (i. e., over 10 lb.) and were normal at the time of delivery (i. e., the urine had been sugar-free). It was postulated that many of these women would ultimately become diabetics, and oral glucose-tolerance tests performed on these subjects at an average interval of three years following delivery disclosed a diabetic configuration in over 30%.[5]

The study was performed with suitable normal subjects, known diabetics, and these so-called prediabetic women (who had given

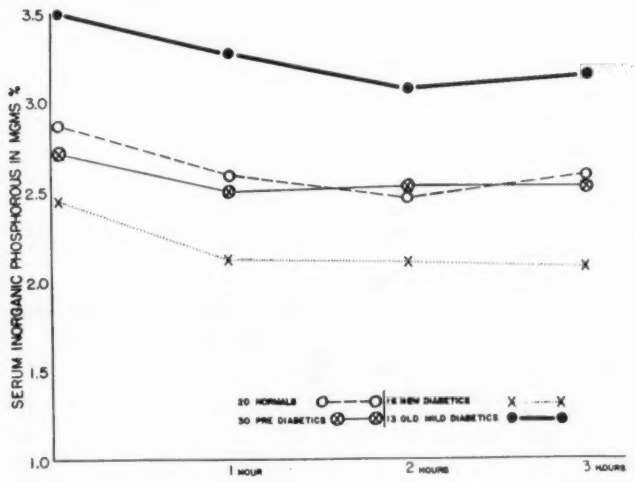


Fig. 1.—Mean change in inorganic phosphorus during oral glucose-tolerance test.

birth to big babies but whose glucose tolerance was still normal). Of prime interest was whether this latter group would show altered phosphorus metabolism prior to the appearance of any abnormalities of the glucose-tolerance curve.

## METHODS AND PROCEDURES

Serum inorganic phosphorus was determined by a modification of the method of Fiske and Subbarow,[6] while a modification of the method of Benedict [7] was employed for the determination of blood sugar.

During the course of a three-hour oral glucose-tolerance test a portion of the blood sample was withdrawn, centrifuged, and 2 cc. of serum mixed immediately with trichloroacetic acid and refrigerated. Within four hours the procedure was completed and the results determined. Thus, fasting and 1-hour, 2-hour, and 3-hour samples were taken in the oral test, while with the intravenous route fasting and 30-, 60-, 90-, 120-, and 150-minute values were determined. All subjects had ingested a liberal diet containing at least 200 gm. carbohydrate for at least two days prior to testing.

The groups were separated as follows:

The experimental group (prediabetics) were 30 women who had each given birth to a large baby but currently exhibited a normal glucose-tolerance curve.

A series of 20 young women of comparable age, nutrition, and social status who were on the gynecology service for diagnostic dilation and curettage were designated as normals.

A group of 16 mothers of big babies whose glucose-tolerance curve was diabetic at the time of testing was considered recent diabetics, since they had been normal three years previously, at the time of delivery.

Thirteen elderly diabetics of long standing who never had required insulin were included in the study and were designated old mild diabetics. The blood glucose values corroborated the clinical diagnoses and will not be listed here (Fig. 1).

TABLE 1.—*Serum Inorganic Phosphate Response to Oral Glucose in Normal Persons and in Patients with Mild Long-Standing Diabetes, Prediabetic Women, and Patients with Recent Diabetes*

| | Serum Inorganic Phosphate After Oral Glucose, Mg./100 Cc. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Normals (20) | | Mild Long-Standing Diabetes Mellitus (14) | | Prediabetic Women (27) | | Recent Diabetes Mellitus (13) | |
| Time, Hr. | Mean | S. D. | Mean | S. D. | Mean | S. D. | Mean | S. D. |
| 0 | 2.86 | ±0.91 | 3.52 | ±0.92 | 2.71 | ±0.71 | 2.48 | ±0.65 |
| 1 | 2.63 | ±0.65 | 3.29 | ±0.75 | 2.49 | ±0.84 | 2.15 | ±0.55 |
| 2 | 2.46 | ±0.66 | 3.06 | ±0.75 | 2.46 | ±0.69 | 2.14 | ±0.70 |
| 3 | 2.59 | ±0.74 | 3.11 | ±0.75 | 2.53 | ±0.56 | 2.06 | ±0.54 |

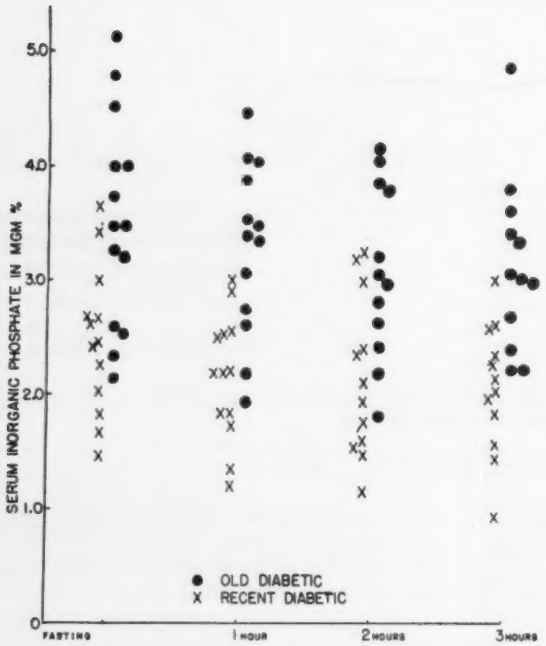


Fig. 2.—Serum phosphate during oral glucose-tolerance test.

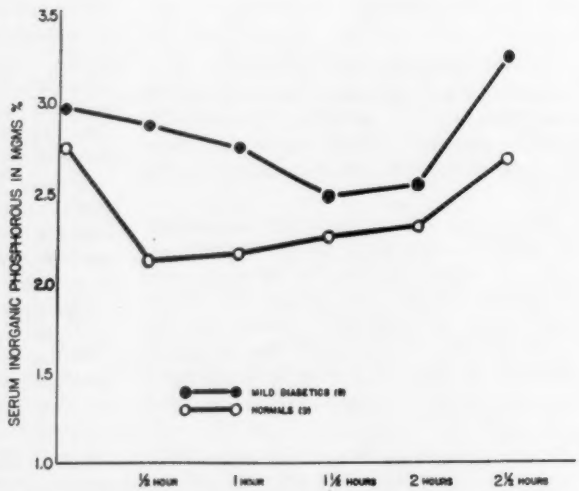


Fig. 3.—Change in inorganic phosphorus during the course of an intravenous glucose-tolerance test.

## RESULTS

Figure 1 shows composite serum inorganic phosphate curves of all four groups during the course of an oral glucose-tolerance test. The mean values and respective standard deviations are listed in Table 1.

Little if any difference is noted between the normals and so-called prediabetic group (normal response to glucose). The mean phosphate values for the 20 normal subjects are 2.86±0.91, 2.63±0.65, 2.46±0.66, and 2.59±0.74, while comparable levels for the prediabetic women are 2.71±0.71, 2.49 ±0.84, 2.46±0.69, and 2.53±0.56. The curves can almost be superimposed one upon the other, and the fasting levels differ but

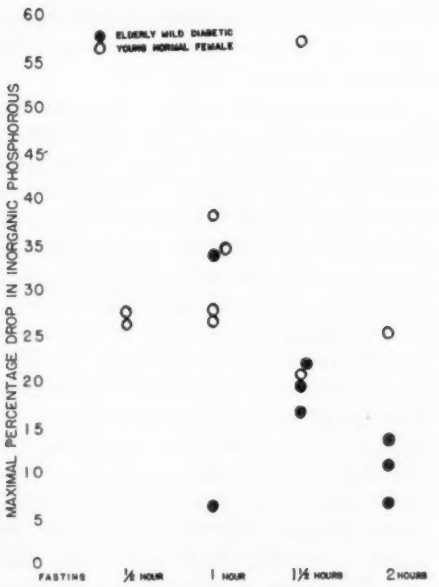


Fig. 4.—Inorganic phosphorus levels during intravenous glucose-tolerance test.

little. The old mild diabetic group shows a higher fasting level and maintains this throughout the procedure, with values of 3.52±0.92, 3.29±0.75, 3.06±0.75, and 3.11 ±0.75 mg. per 100 cc. Of interest and significant difference is the curve of the recent diabetic group, in which the phosphorus values are moderately below the normal but much lower than those of the older diabetic group. The recent diabetics gave values of 2.48±0.65, 2.15±0.55, 2.14±0.70, and 2.06 ±0.54 mg. per 100 cc. (Fig. 2).

Figure 2 presents the data as a "scattergram" and emphasizes the marked differences between the two groups, which emerged even more graphically from a statistical study in which the differences between means of comparable values in the two groups yielded values of *P* less than 0.01 in each of the four determinations.

Figure 3 represents the mean inorganic serum phosphorus values following the intravenous administration of glucose, and these are for the normals 2.86±0.14, 2.13±0.12, 2.14±0.12, 2.21±0.12, 2.29±0.33, and 2.42 ±0.42; for the mild diabetic the values are 2.99±0.66, 2.89±0.56, 2.73±0.73, 2.48±0.46, 2.56±0.63, and 3.24±0.53. The normal shows an earlier drop and to a somewhat greater extent than does the diabetic. Figure 4, which plots the maximal percentage drop in phosphorus against the time during the test at which it occurred, shows more clearly that the fall occurs later in the diabetic group, but from the data at hand the statistical significance is not clear-cut. This is somewhat at variance with the work of Forsham and Thorn, as will be noted in the discussion to follow.

Table 4 lists the mean values for the maximum drop and the mean time at which it occurred. With the oral route these are for the normals 17.5% and 2.1 hours, for the long-standing mild diabetics 18.4% and

TABLE 2.—*Serum Inorganic Phosphate Response to Intravenous Glucose in the Normal and in Mild Long-Standing Diabetes Mellitus*

| | Serum Inorganic Phosphate After Intravenous Glucose, Mg./100 Cc. | | | |
|---|---|---|---|---|
| | Normals (9) | | Diabetes Mellitus (8) | |
| Time, Min. | Mean | S. D. | Mean | S. D. |
| 0..... | 2.86 | ±0.14 | 2.99 | ±0.66 |
| 30..... | 2.13 | ±0.12 | 2.89 | ±0.56 |
| 60..... | 2.14 | ±0.12 | 2.73 | ±0.73 |
| 90..... | 2.21 | ±0.12 | 2.48 | ±0.46 |
| 120..... | 2.29 | ±0.33 | 2.56 | ±0.63 |
| 150..... | 2.42 | ±0.42 | 3.24 | ±0.53 |

TABLE 3.—*Statistical Examination of the Difference Between Mean Values of Inorganic Phosphate During Oral Glucose-Tolerance Test in Long-Standing and Recent Diabetes*

| | Phosphate, Mg./100 C. | | | | | |
|---|---|---|---|---|---|---|
| | Long-Standing Diabetes (14) | | Recent Diabetes (13) | | | |
| Time, Hr. | Mean | S. D. | Mean | S. D. | *t* | *P* |
| 0..... | 3.52 | ±0.92 | 2.48 | ±0.65 | 3.376 | <0.01 |
| 1..... | 3.29 | ±0.75 | 2.15 | ±0.55 | 4.41 | <0.01 |
| 2..... | 3.06 | ±0.75 | 2.14 | ±0.70 | 3.08 | <0.01 |
| 3..... | 3.11 | ±0.75 | 2.06 | ±0.54 | 4.05 | <0.01 |

2.5 hours, for the prediabetic women 19.4% and 1.7 hours, and for the recent diabetics 23.3% and 2.3 hours. When the intravenous test was employed these respective values were 31.5% and 1.1 hours for the normals and 16.2% and 1.6 hours for the diabetics.

Obviously the mean figure for the time at which maximum drop is noted can have no actual validity per se, inasmuch as it represents an average of absolute time intervals following the start of the test and not a definitive time at which a specific blood sample was drawn (e. g., 2.1 hours after start); nevertheless, this mean conveys a distinct impression of the possibility that the maximal fall in serum inorganic phosphorus may occur earlier or later in certain groups.

TABLE 4.—*Maximum Drop in Serum Inorganic Phosphate Following Glucose Administration*

| | Patients, No. | Maximum Drop of Phosphate, per Cent | Mean Time, Hr. |
|---|---|---|---|
| Oral glucose-tolerance test | | | |
| Normals | 19 | 17.5 | 2.1 |
| Long-standing diabetics | 12 | 18.4 | 2.5 |
| Prediabetic women | 24 | 19.4 | 1.7 |
| Recent diabetics | 13 | 23.3 | 2.3 |
| Intravenous glucose-tolerance test | | | |
| Normals | 9 | 31.5 | 1.1 |
| Diabetics | 8 | 16.2 | 1.6 |

When expressed as a function of the route of administered glucose, the difference in response between the oral and intravenous routes seems highly interesting and will be considered further.

## COMMENT

Previous experiments have demonstrated that the serum inorganic phosphorus is involved in carbohydrate metabolism.† The decrease in urinary phosphate excretion and the drop in serum phosphorus following glucose or insulin have helped point up the relationship of phosphorus to the metabolism of glucose.[9] Pollack's classic experiment of perfusing the hind limb of the dog is interpreted as fixing the peripheral musculature as the site of interaction of the drop in serum phosphate and active entry of glucose into the glycolytic cycle.[1]

The results of our studies confirm much of the work previously published.‡ Undoubtedly the serum phosphorus changes were influenced by the administration of glucose, and there is an obvious difference in curves between the normal and the diabetic following intravenous glucose, the drop in phosphorus being greater and more abrupt in the normal. An interesting observation is the time interval for a maximum fall in phosphorus in the various groups. Here the difference between normal and diabetic is also definite but still could not be applied to a single individual with any validity. Thus, while group differences are striking, individual overlapping within groups makes the phosphate response a test of doubtful significance for the single case. Discussion of the reason for the difference (i. e., as a mirror of peripheral utilization of glucose) has been mentioned in other reports [3] and will be further elaborated below.

The wide divergence between the phosphorus curve of the old diabetics and that of the recent diabetics has been mentioned. Although of considerable statistical significance, the theoretical implications of this finding are not immediately apparent and will not be dealt with in this paper.

Following intravenous administration of glucose a distinct difference is noted between the serum inorganic phosphate curve of the diabetic and that of the normal. Of extraordinary interest is the abolition of this difference between the normal curve and the diabetic curve when glucose is administered by mouth. The data reveal that following oral glucose the serum phosphate curve of the normal approaches that of the diabetic in that the pronounced initial drop seen with the former intravenous glucose is no longer apparent.[11] The explanation for this is largely conjectural. When the oral route is employed two new factors are introduced to affect the metabolism of glucose prior to its reaching

† References 1-4.

‡ References 10 and 11.

the peripheral tissues, namely, the intestinal wall and the liver. Verzar described a mechanism in which phosphorus is necessary to transport glucose across the intestinal mucous membrane, and it has never been noted that this operates differently in the diabetic than in the normal.[12] Therefore one is forced to conclude that some metabolic process in the liver accounts for the abolition of the differences noted when the intravenous route is employed. Furthermore, this evidence indirectly implicates the peripheral tissues as the site of the fall in serum inorganic phosphorus in the normal during glucose metabolism and tends to substantiate the fundamental work of Pollack and co-workers.[1] The role of the liver in this mechanism can probably be better elucidated by work with a simpler system, such as the eviscerated animal, liver slices, hepatic venous catherization, etc.

## SUMMARY AND CONCLUSIONS

A distinct difference exists between the phosphorus curves of normals and of diabetics after the intravenous administration of glucose.

A greater and earlier drop in phosphate is noted in the normal as compared with the diabetic.

Since considerable overlap occurs between the two groups, this procedure is inapplicable for individual patients but is still statistically valid for groups.

The above-mentioned difference is not seen when glucose is administered to normals and diabetics by the oral route. The results also disclose no differences in the phosphate curves of normals and prediabetic women.

Following oral glucose, the marked drop noted in the normal phosphorus curve with the intravenous route is absent and the curve of the normal more closely approximates that of the diabetic.

It is postulated that after oral glucose the interposition of the liver abolishes the phenomenon noted in the normal when the intravenous route is employed, and this indirectly implicates the peripheral tissues as the site of phosphate utilization.

A more thorough elucidation of the role of the liver in phosphorus metabolism awaits work with simpler systems.

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# Comparative Incidence of Cholelithiasis in the Negro and White Races

**A Study of 6185 Autopsies**

JOSEPH A. CUNNINGHAM, M.D.
and
FIRMON E. HARDENBERGH, B.S., Birmingham, Ala.

According to the latest census (1950), the population of Jefferson County, Ala., which includes the city of Birmingham, is 558,928. There are basically two racial groups represented, namely, a white segment (59.1%), predominantly of European origin, and a Negro segment (40.9%), of African origin. (The state of Alabama as a whole shows a ratio of 61.8% whites to 38.2% Negroes.) In order to evaluate the health problems of this area properly it is important to delineate the disease patterns of these two races. As a part of this larger project we undertook to investigate the over-all incidence of cholelithiasis in Alabama and, more specifically, to establish the comparative incidence of this disease in the two races.

## MATERIAL

The material for this study was obtained from the routine autopsy service of five Alabama hospitals, four of them in Jefferson County. Three of these four, namely, St. Vincent's, Lloyd Noland, and the Carraway Methodist, are general hospitals with an average bed capacity of 250 each and have varying percentages of white and Negro patients. The fourth hospital, the Jefferson-Hillman, is a 600-bed unit of the Medical College of Alabama, with an approximate 60:40 ratio of white to Negro patients. The fifth hospital surveyed was the Veterans Hospital in Tuskegee, Ala., which admits only adult male Negroes.

From these five sources we were able to collect for study 6185 consecutive autopsies covering a period from 1946 to 1953. We arbitrarily eliminated a small number of cases in which the necessary information was missing. This series represents a fair sample of the patients, from all economic levels, seen in general hospitals in Alabama.

For purposes of analysis the 6185 autopsies were divided into two groups: the white, totaling 2913 and the Negro, totaling 3272. They were then subdivided into age and sex groups. Table 1 summarizes the age, sex, and racial incidence of the entire series. (We made no attempt to classify the stones into the usual four types—radiate cholesterol, compound cholesterol, calcium-bilirubinate, and mixed stones—because in most of the protocols the description of the stones was not detailed enough for accurate typing and in none of them were the stones chemically analyzed.)

## RESULTS OF STATISTICAL STUDY

In the entire series there were 341 patients with cholelithiasis, an over-all percentage incidence of 5.51. In Table 2 the cases of cholelithiasis are broken down into race, sex, and age groups, with the absolute fraction and the percentage of each group. Table

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From the Department of Pathology, University of Alabama Medical Center.

Read before the Section on Pathology and Physiology at the 104th Annual Meeting of the American Medical Association, Atlantic City, June 8, 1955.

TABLE 1.—*Age, Sex, and Racial Incidence in 6185 Autopsies*

| Racial Groups | Birth-20 Yr. | | 21-40 Yr. | | 41-60 Yr. | | 60 Yr. and Over | | Totals | | Grand Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | |
| White | 407 | 287 | 177 | 148 | 571 | 283 | 679 | 361 | 1834 | 1079 | 2913 |
| Negro | 457 | 393 | 358 | 159 | 1109 | 222 | 461 | 113 | 2385 | 887 | 3272 |
| Totals | 864 | 680 | 535 | 307 | 1680 | 505 | 1140 | 474 | 4219 | 1966 | 6185 |

TABLE 2.—*Age, Sex, and Racial Incidence of Cholelithiasis in 341 Autopsy Cases*

| | Birth-20 Yr. | 21-40 Yr. | 41-60 Yr. | 60 Yr. and Over | Totals |
|---|---|---|---|---|---|
| White male | 1/407 (0.245)* | 3/177 (1.70) | 44/571 (7.70) | 89/679 (13.10) | 137/1834 (6.92) |
| White female | 0/287 (0) | 10/148 (6.75) | 33/283 (11.66) | 77/361 (21.35) | 120/1079 (11.12) |
| Negro male | 0/457 (0) | 2/358 (0.558) | 21/1109 (1.89) | 18/461 (3.90) | 41/2385 (1.71) |
| Negro female | 0/393 (0) | 10/159 (6.28) | 16/222 (7.70) | 17/113 (15.04) | 43/887 (4.84) |
| Totals | 1/1544 (0.064) | 25/842 (2.97) | 114/2185 (5.21) | 201/1614 (12.44) | 341/6185 (5.51) |

* Ratio of cases of cholelithiasis to total cases in each group; percentage is given in parentheses.

3 summarizes the racial incidence calculated (1) for the entire series and (2) for the age groups above 20 years. From these two Tables it can be seen that the white race is approximately four times as susceptible to cholelithiasis as the Negro race, the percentage incidence being 8.82% white to 2.56% Negro. It is also clear that the Negro male is the least likely to develop this disease. Equally striking is the consistent sex difference, with the percentage incidence significantly higher in the female of both races and with the highest incidence of cholelithiasis found in the white female. It is interesting to note how, for practical purposes, this sex difference equalizes the race factor when the incidence in the white male and in the Negro female are compared.

TABLE 3.—*Total Racial Incidence of Cholelithiasis in 341 Autopsy Cases*

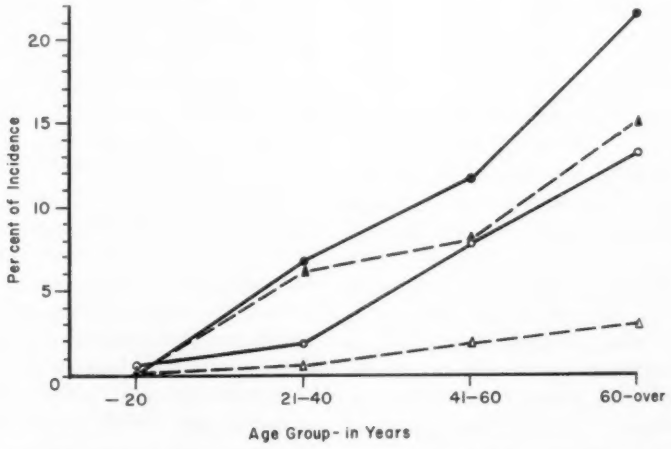
| Race | All Cases | Cases Over 20 Yr. (340) |
|---|---|---|
| White | 257/2913 (8.82)* | 256/2219 (11.53) |
| Negro | 84/3272 (2.56) | 84/2422 (3.46) |
| Total | 341/6185 (5.51) | 340/4641 (7.32) |

* Ratio of cases of cholelithiasis to total cases in each group; percentage is given in parentheses.

The Figure shows the percentage incidence for the different age groups. This graph clearly demonstrates the role of age in this disease. In the white male and female there is a significant rise with advancing age. This same rise is also noted in the Negro female but is practically absent in the case of the Negro male.

## COMMENT

A tabulation of statistical reports in the literature on the comparative racial incidence




Race, sex, and age-group incidence of cholelithiasis. Open circle, white male; black circle, white female; open triangle, Negro male; black triangle, Negro female.

of cholelithiasis (based on 113,503 autopsies, with ours included) is shown in Table 4. These data support our finding that cholelithiasis is less frequent in the Negro than in the white race and also suggest that the incidence in other pigmented races is correspondingly low.

The low incidence of cholelithiasis in the Negro was noted in the literature as early as the latter part of the 19th century. In 1908 Rodman,[1] writing in Keen's "Surgery,"

TABLE 4.—*Comparative Racial Incidences of Cholelithiasis on the Basis of Autopsy Reports*

| | Total Autopsies | Cholelithiasis | Per Cent Incidence |
|---|---|---|---|
| **White Race** | | | |
| Americans | | | |
| Baltimore | 1,018 | 80 | 7.80 |
| Birmingham | 2,913 | 257 | 8.82 |
| Chicago | 1,473 | 48 | 3.26 |
| Cleveland | 1,785 | 162 | 9.08 |
| California | 3,000 | 213 | 7.10 |
| Philadelphia | 13,088 | 1,832 | 13.30 |
| Austrians | 19,974 | 1,557 | 7.80 |
| Germans | 1,150 | 141 | 12.26 |
| Swiss | 16,025 | 1,714 | 10.70 |
| British | 20,851 | 835 | 4.00 |
| Totals | 81,227 | 6,839 | 8.42 |
| **Other Races** | | | |
| Americans (Negro) | | | |
| Atlanta | 696 | 2 | 0.29 |
| Baltimore | 634 | 34 | 5.50 |
| Birmingham | 3,272 | 84 | 2.56 |
| Chicago | 122 | 2 | 1.64 |
| Cleveland | 370 | 20 | 5.41 |
| Philadelphia | 7,913 | 394 | 4.98 |
| Bantu | 1,677 | 50 | 2.98 |
| Canal Zone | 1,088 | 24 | 2.21 |
| Trinidad | 7,557 | 5 | 0.07 |
| Chinese | 266 | 6 | 2.25 |
| Japanese | 8,406 | 268 | 3.07 |
| Korean | 275 | 8 | 2.91 |
| Totals | 32,276 | 887 | 2.75 |

reported that in 15 years of surgical practice in Louisville neither had he seen a single clinical case of cholelithiasis in a Negro nor had there been an autopsy report of stones. Gould and Warren[2] reported (1905) from the same city that only 1 of 106 patients presenting stones at surgery had been a Negro. They failed, however, to report the relative number of Negroes and whites undergoing surgery. Clark,[3] from the Canal Zone, in 1914, reported only 24 cases of stones in 1088 consecutive autopsies of male West Indian Negro workers. He attempted to account for this incidence of 2.21% (which he considered high) by calling attention to the prevalence of colitis, enteritis, and malaria, and (recalling Naunyn's theory that "infection leads to stones") it should be noted that most of the subjects in his series were fairly young Negro males; of all the age-sex-race combinations young Negro males consistently show the lowest incidence of cholelithiasis. In 1918 Mitchell[4] reported the results of a study of 1600 coroner's cases in Chicago: of 85 Negro males not one presented stones, and of 37 Negro females 2 had stones (5.41%); of the 1225 white males 28 had stones (2.29%), as did 20 of the 248 white females (8.07%). Mitchell's figures show total male and female incidence as follows: male, 28 in 1315 (2.1%), and female, 22 in 285 (7.72%).

Bloch,[5] in 1926, wrote that in 18½ years on the Negro surgical service at Charity Hospital in New Orleans only 2 cases of cholelithiasis had been seen in Negro males and 15 had been reported in Negro females. In support of Bloch's findings is a more recent report (1948) of Windley,[6] again from Charity Hospital in New Orleans. He states that in a series of 1,409,603 hospital admissions (738,271, or 52.4%, white and 671,361, or 47.6%, Negro) a total of 1538 cases of cholelithiasis was demonstrated by radiology, surgery, or autopsy. This is a total incidence of 0.11%. Of these 1538 cases, 1188 (77.3%) were white and 350 (22.7%) were Negro. These figures reveal that 0.16% of the white admissions presented cholelithiasis, while stones were found in only 0.05% of the Negro admissions.

In 1927 Alden,[7] from Atlanta, reported 2 cases of stones in 696 Negro autopsies, but no mention was made of sex ratio. He also stated that, of 23,016 admissions to the Negro service, 39 cases were diagnosed as cholecystitis or cholelithiasis, and in only 9 of these were stones later demonstrated. This is equivalent to an incidence of 0.04% as compared with 0.05% in New Orleans.

Perhaps the most complete record and analysis of the incidence of cholelithiasis in

the American Negro at autopsy was recorded by Ludlow [8] in 1930. For a basis of comparison of the incidence of stones in Koreans, Ludlow made a study of 2800 Cleveland autopsies—366 Negroes over 20 years of age and 1785 whites over 20. The total incidence of stones in these 2151 adults was 6.54% (183 cases). Of the 1161 white males 84 (7.24%) had stones; of the 624 white females, 78 (12.5%); of the 202 Negro males, 8 (3.96%), and of the 164 Negro females, 12 (7.31%) presented stones.

In 1932 Jaffe,[9] from Cook County in Chicago, reported the incidence in 2621 consecutive autopsies on subjects over 20, the total number of autopsies in the series being 3633. Total incidence of stones was 9.03%: that in white males, 6.76%; in white females, 17.57%; in Negro males, 1.04%, and in Negro females, 10.23%. Unfortunately, the author failed to give absolute numbers of cases in his incidence report.

Lopis,[10] in 1947, reported a study of 3793 European, Bantu, and Eurafrican autopsies in Johannesburg, South Africa. Of the 1099 Bantu males 17 (1.5%) had stones, and of the 578 Bantu females 33 (5.7%) had stones. These percentages are very close to those we found for Negroes in Alabama. Lopis found that cholelithiasis was four times as prevalent in Europeans in Johannesburg as in the Bantu there. His most interesting finding, however, was the preponderance of pigmented stones in the Bantu (50%-70%) as compared with the preponderance of mixed stones in Europeans (50%-60%). Jaffe had concluded that there is no significant statistical difference between the types of stones found in Negroes and whites in Chicago.

In 1952 Becker and Chatgidakis,[11] from Johannesburg, reported a study of 9500 consecutive autopsies on subjects representing the main racial groups on the Witwatersrand, namely, Europeans, Bantu, and Eurafricans. (Some of his material from the Johannesburg General Hospital is the same as that of Lopis.) In the 9461 cases suitable for analysis, stones were found in 699 (7.39%). There were 4416 European whites and 4494 Bantu in his series, and of these 2760 were white males; 1656, white females; 2799, Bantu males, and 1695, Bantu females. The percentage incidence of cholelithiasis for the different sex and race groups is as follows: white male, 9.97%; white female, 19.52%; Bantu male, 0.96%; Bantu female, 3.83%. His total incidence for the white South African is 13.52%, and that for the Bantu South African, 2.04%. An interesting feature of this report, as in that of Lopis, is the breakdown of the gallstones. This breakdown is based upon the physical appearance of the stones, no chemical analyses being done. Of the four types (radiate cholesterol, compound cholesterol, calcium-bilirubinate, and mixed stones) Becker found that the multiple, facetted, mixed stone is the commonest calculus in the female of both races and is the stone largely responsible for the increased incidence of cholelithiasis in this sex. He also refutes Lopis' claim that the pigment stone is the predominant one in the colored races. His figures show that this apparent prevalence is due to the rarity of the other types of gallstones. In fact, his tables show that there is no significant difference in sex incidence and that the calcium-bilirubinate stone is actually slightly commoner in the white race.

In summarizing the confusing literature we feel safe in stating that in all reports the incidence of gallstones is higher in the white race than in the Negro and is highest in white females (12-17%) and lowest in Negro males (1-3%) and that generally the incidence in white males and Negro females is approximately the same at some midpoint (6%-10%) between the two extremes noted above. Thus it appears that our own findings coincide with those from other parts of the world.

As regards the etiology and pathogenesis of cholelithiasis, the literature, in the words of Rous [12] and his co-workers, is "top-heavy with hypothesis and dismal with uncorrelated observations." There is, however, an area of fairly general agreement among investigators, and it would appear worth while at this point to delineate it. It is, for example, gen-

erally conceded that the solitary pure cholesterol stone represents an aseptic formation most likely due to a metabolic disturbance, with a shift of the bile salt-cholesterol ratio. Furthermore, most investigators agree that the calcium-bilirubinate stone, so frequently associated with diseases (such as the hemolytic anemias) which produce an increased concentration of bilirubin in bile, represents an aseptic precipitation of this pigment. The mixed stones, which are often multiple and facetted, are considered by most to be at least associated with infection, if not directly caused by the degradation of the chemical composition of bile resulting from it. Hence it would appear that there is some agreement that no single hypothesis will explain the development of all types of gallstones and that a different mechanism of formation is operative for each type of stone. There is far less agreement on the importance of the role of such factors as stasis, pregnancy, obesity, diabetes, diet, etc. And, although the well-documented statistical studies in the world literature leave little room for doubting the significance of the factors of race, sex and age, there is certainly a need for further study on how these factors exert their influence.

## SUMMARY AND CONCLUSIONS

A study of the comparative incidence of cholelithiasis in the Negro and white races, based upon 6185 consecutive autopsies performed in Alabama during the period of 1946-1953, inclusive, is reported.

The results indicate that cholelithiasis is found approximately four times as frequently in the white race as in the Negro and that the highest incidence is found in the white female (11.12%), the lowest in the Negro male (1.71%). The importance of the sex factor is evidenced by the significantly higher incidence in the female of both races. Also clearly shown is the role of age in this disease—with advancing age, a steady rise in incidence is seen in the white male and female; it is also noted in the Negro female but is practically absent in the case of the Negro male.

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# The Rehabilitation of Hemiplegics with Auricular Fibrillation

**The Effect of Conversion to Regular Sinus Rhythm; A Preliminary Report**

JACK SOKOLOW, M.D.
LAWRENCE I. KAPLAN, M.D.
and
HOWARD A. RUSK, M.D., New York

In recent years much of the literature has been devoted to the magnitude of the problem of the cardiac patient. The treatment and growing number of cardiacs who are partially disabled as a result of myocardial infarction, decompensation, etc., have been viewed with growing concern by physicians throughout the nation.

Of 61,000 people rehabilitated in 1953 using the services of the State vocational rehabilitation agencies, 4.3% were cardiacs as opposed to 10.9% with tuberculosis, on the basis of a return to gainful employment or improved work status.

In 1952 Benton and Rusk [1] stated that 90% of hemiplegics can be retrained and, of these, about one-third can be productively placed. The enormous importance of effective rehabilitation becomes obvious in the light of these statistics.

The experiences in the Work Classification Unit at Bellevue Hospital in New York City have shown that cardiacs can work, and Benton and Rusk have further stated that cardiac kitchen energy cost studies indicate that "the compensated cardiac woman is no less efficient in the performance of these activities than the non-cardiac." [2]

However, in rehabilitation these patients with severe cardiac disease coupled with an obvious disability such as hemiplegia, paraplegia, or amputation are called upon to perform a certain amount of physical activity; they must be closely observed in terms of cardiac reserve. This is of the utmost importance in disabled patients who also suffer from auricular fibrillation, since the decrease in cardiac reserve becomes marked as they perform the various activities and exercises necessary for their rehabilitation. As a result this type of patient presents a real problem.

"Moderate exertion usually causes a greater acceleration of the heart rate if it is fibrillating than it does when sinus rhythm is present. This causes decreased efficiency and palpitation." [3]

Jezer states that "the work experience of rheumatic cardiacs who show auricular fibrillation is very poor and their rate of absenteeism is much higher [than cardiacs with minor arrhythmias], because this arrhythmia is usually associated with advanced heart disease, congestive heart failure, and frequently peripheral embolization." [4]

Furthermore, the studies of Kory and Meneely using cardiac catheterization have shown that fibrillating patients compensated by all possible means except quinidine have low resting and exercise cardiac output but that after conversion to regular sinus rhythm the output is increased during rest and exercise.[5] In addition, other work has shown

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New York University-Bellevue Medical Center, Institute of Physical Medicine and Rehabilitation; U. S. Public Health Fellows (Dr. Sokolow and Kaplan) and Director, Institute of Physical Medicine and Rehabilitation, and Professor, Physical Medicine and Rehabilitation (Dr. Rusk).

that cardiac reserve on exercise is greatly improved on conversion of auricular fibrillation to regular sinus rhythm but that it is not greatly changed at rest. This same study, by Hecht, Osher, and Samuels, indicated that of 24 patients converted there were none who suffered embolic phenomena after conversion.[6]

Other authors have also stated that the incidence of emboli in the patient with unconverted fibrillation is much greater than when the heart beat has been converted to regular sinus rhythm.[7] In the series of McMillan and Welfare and in M. Sokolow's series of 72 patients no emboli occurred with or immediately after conversion. However, in Goldman's series of 80 patients there were 3 episodes of embolism during conversion, but he states that 27.5% of patients had a history of embolism while in auricular fibrillation. His conclusion is that the chances are seven to one in favor of a patient suffering an embolism while in auricular fibrillation as compared with the likelihood of occurrence at the time of conversion to regular sinus rhythm.[8]

We, therefore, felt that since the cardiac patient in rehabilitation is often faced with the necessity for carrying on physical activity in order to achieve a goal of independence, and since the danger of embolism has been shown to be greater during fibrillation than during conversion or after, the patient's performance might be increased by conversion of the auricular fibrillation to normal sinus rhythm. This study was begun in order to determine whether such treatment would improve the efficiency of the patient in a program of rehabilitation.

## METHOD

With the above studies in mind, we used as formal indications for conversion from fibrillation to regular sinus rhythm a history of one or more embolic episodes and/or persistent congestive heart failure. It was, therefore, expedient to use patients who were hemiplegic as a result of an embolic episode.

As a method of quinidine administration, we followed the routine suggested by M. Sokolow, in which a given amount of quinidine is given every two hours for five doses daily, the amount per dose being increased daily until conversion is achieved.*

Our first step was to procure an ECG to prove the presence of auricular fibrillation. The patient continued on the program of activities prescribed for rehabilitation (therapeutic exercise, ambulation, training in activities of daily living, etc., depending on individual needs), and we studied the venous pressure and circulation times (dehydrocholic acid N. F. [Decholin] and ether times). We also evaluated the patient's tolerance to exercise, checking the pulse and ventricular rates, blood pressure, and respiration before and after exercise. Before exercise, the patient was resting in a wheelchair. For exercising the patient, we used a standard set of six steps, each 6 in. high, and the patient was asked to ascend and descend twice in as close to two minutes as possible.

In one case the patient was relatively able and an additional test, of going up and down a 4 in. step to fatigue level, was used. This is, of course, a subjective measurement.

After these determinations, we attempted conversion to regular sinus rhythm using quinidine as follows:

| | Quinidine, Gm. P. O. Q. 2 H. for 5 Doses |
|---|---|
| First day | 0.2 |
| Second day | 0.4 |
| Third day | 0.6 |
| Fourth day | 0.8 |
| Fifth day | 1.0 |

During the period of conversion the patient was kept on bed rest.

If there was no conversion after the fifth day, we admitted failure to convert.

Where nausea and vomiting were the only toxic signs, we used belladonna to control these symptoms. When the patients were not followed by close regular ECG (week ends) they were maintained on 0.4 gm. or 0.6 gm. of quinidine q. i. d. until regular ECG coverage and regular dosage were reinstituted.

After conversion to regular sinus rhythm we determined again the venous pressure and circulation times and the tolerance to exercise as described above.

## DATA

Four cases were studied to give us a preliminary idea of whether further work was indicated.

Case 1.—A 32-year-old white woman was admitted in 1952 with a history of rheumatic fever in 1948. There was no previous history of rheumatic fever and no other pertinent medical history.

* References 9 and 10.

Rheumatic heart disease was diagnosed in 1948. Between March, 1950, and August, 1951, she suffered two "strokes" (the second of which left her with right hemiplegia and partial aphasia) and two pulmonary infarcts. In January, 1952, a therapeutic abortion was done, and the month following she had an episode of acute heart failure.

In March, 1952, she was admitted for rehabilitation, and physical examination revealed slight aphasia and spastic right hemiparesis with stiffness of the right shoulder and limited range of motion. Examination of her cardiovascular status showed rheumatic heart disease with enlarged heart, aortic insufficiency, mitral stenosis and insufficiency, and regular sinus rhythm. She was on a salt-free diet and digitoxin, 0.2 mg. daily.

A program of mild activities of daily living and sedentary occupational therapy was prescribed, but about one week later the patient developed subclinic near her home in August, 1952. We have learned that she died in February, 1953, but the circumstances are not known.

This case gave rise to the interest in this study, because of the marked increase in efficiency after conversion.

CASE 2.—A 35-year-old Negro woman was admitted for rehabilitation June, 1953, with a diagnosis of rheumatic heart disease, auricular fibrillation (discovered February, 1953), mitral stenosis and insufficiency, and enlarged heart. She had had an embolic episode in February of that year, which had resulted in right hemiparesis and partial aphasia.

On a training program she had improved to the point where she could ambulate for short periods with assistance or a cane and if given enough time could carry out her activities of daily living. She was discharged in August but was readmitted in September, 1953, because she was not able to

TABLE 1.—*Before Conversion**

| Case No. | | Pulse Rate | Ventricular Rate | Blood Pressure | Respiration Rate | Venous Pressure, Mm. | Dehydrocholic Acid, Sec. | Ether, Sec. |
|---|---|---|---|---|---|---|---|---|
| 2 | Resting | 102 | 140 | 150/95 | 16 | 142 | 20 | 10-12 |
| | Exercise | 110 | 172 | 170/100 | 16-18 | | | |
| 3 | Resting | 116 | 136 | 150/90 | 20 | | | |
| | Exercise | 136† | 136 | 160/95 | 24 | 110 | 15 | 9 |
| | | 150‡ | 150 | 155/85 | .. | | | |
| 4 | Resting | 78 | 100 | 128/90 | 20 | 108 | 12 | 6 |
| | Exercise | 84 | 100 | 145/95 | 28 | | | |

* Return to resting levels ranged between one and one-half and two minutes.
† Six-inch step exercise.
‡ Four-inch step exercise (up and down to patient's fatigue level) 11 times.

acute bacterial endocarditis. She was treated with penicillin and returned for rehabilitation after three months. Some time later an ECG showed auricular fibrillation, and conversion to regular sinus rhythm was carried out by giving quinidine 0.2 gm. p. o. hourly for three doses and then 0.4 gm. for a fourth dose. (It was after this case that we adopted the exercise procedure and method of quinidinization.) An ECG taken an hour after the last dose showed regular rhythm at 100 per minute. A maintenance dose of 0.4 gm. four times daily was given for one day, and then the dosage was reduced to 0.2 gm. three times daily. The digitoxin was reduced to 0.1 mg. daily, and the pulse rate slowed to 80 per minute and was regular.

The subjective improvement was marked, and her physical activity was increased. As a result she did very well, being able to ambulate with a short leg brace. However, her digitoxin had to be increased to 0.1 mg. and 0.2 mg. on alternate days.

She was practically self-sufficient in dressing, undressing, toilet activities, bathtub, etc., and in ambulation, and was discharged to an outpatient

function well enough to run the household and care for her child.

Physical examination showed that she was gradually developing cardiac decompensation and that she was not on digitalis. How long before February, 1953, the auricular fibrillation had existed was unknown.

She was digitalized and maintained on digitoxin 0.1 mg. daily and was on a salt-free diet. She resumed a program of ambulation, therapeutic exercise, occupational therapy to improve her coordination, and concentrated training in activities of daily living. She showed slow but steady progress under this regimen, and it was then decided to convert the auricular fibrillation to regular sinus rhythm in an attempt to improve her efficiency and speed up her rate of progress.

The venous pressure and circulation times were tested, as was her tolerance to exercise (Table 1).

The next day quinidinization was begun and carried out as follows:

First day, Quinidine 0.2 gm. q. 2 h. for 5 doses
Second day, Quinidine 0.4 gm. q. 2 h. for 5 doses
Third day, The patient had regular rhythm as shown by ECG; PR = VR = 74.

She was given a maintenance dose of 0.4 gm. of quinidine q. i. d. for four days, after which it was reduced to 0.2 gm. q. i. d. Six days later reversion to fibrillation occurred, and she was again given quinidine 0.4 gm. q. 2 h. for five doses. Conversion to regular sinus rhythm occurred during the night, and, with a maintenance dose of 0.4 gm. quinidine q. i. d., the rhythm has been regular since.

During the first period of regular rhythm the hemodynamics and exercise tolerance were rechecked (Table 2). The patient felt better subjectively and functioned more efficiently: she carried out her program activities more easily and had a higher fatigue threshold.

CASE 3.—A 55-year-old white woman was admitted for rehabilitation with a diagnosis of pernicious anemia, arteriosclerotic heart disease, auricular fibrillation, mitral insufficiency, and enlarged heart. In May, 1953, she had suffered a right hemiparesis and aphasia (which partially cleared subsequently) as a result of a cerebrovascular episode which was thought to be due to an embolism.

There was a history of heart disease for 10 years prior to admission, and she was being followed in the outpatient clinic when she developed the right hemiparesis. The patient was on a salt-free diet, digitoxin 0.1 mg. daily, ammonium chloride, and meralluride (Mercuhydrin) sodium 2 cc. once weekly. It was known that in September, 1945, her cardiac rhythm was regular, but on examination in December, 1949, auricular fibrillation was discovered. Her history also revealed several episodes of acute pulmonary edema.

In August, 1953, she came for rehabilitation and her digitoxin was increased to 0.2 mg. daily. A program of strengthening exercise for her paretic extremities, ambulation with a brace, occupational therapy to increase hand function, and activities of daily living to obtain maximum self-sufficiency was prescribed. Progress was slow, and it was decided to attempt conversion to regular sinus rhythm.

Her venous pressure and circulation times and exercise tolerance (in this case we used an additional test) were checked (Table 1). Conversion was then attempted:

| | Quinidine, Gm. Q. 2 H. for 5 Doses |
|---|---|
| First day | 0.2 |
| Second day | 0.4 |
| Third day | 0.6 |

Conversion to regular rhythm occurred during the night, and the next day a maintenance regimen of 0.4 gm. q. i. d. was begun. The exercise tolerance and hemodynamics were tested again (Table 2).

About three weeks later an ECG revealed that the heart was again fibrillating, probably, it was felt, because the maintenance dose was too low. The patient received 0.6 gm. of quinidine q. 2 h. for five doses, with reconversion to regular rhythm, and was kept on a maintenance dose of 0.6 gm. q. i. d.

There was subjective improvement, and the program was carried out more effectively, with a significant increase in the rate of progress.

CASE 4.—A 65-year-old white woman was admitted in November, 1953, with a diagnosis of arteriosclerotic heart disease, auricular fibrillation, mitral insufficiency, and enlarged heart. In June, 1952, she suffered an embolic episode which resulted in left hemiparesis. She had first been digitalized in February, 1950.

A program designed to improve her activities of daily living and ambulation was prescribed. Progress was minimal, and the patient became excessively fatigued very easily. It was, therefore, decided to attempt conversion.

As in our previous cases, we studied the hemodynamics and exercise tolerance (Table 1) and

TABLE 2.—*After Conversion**

| Case No. | | Pulse Rate | Ventricular Rate | Blood Pressure | Respiration Rate | Venous Pressure, Mm. | Dehydrocholic Acid, Sec. | Ether, Sec. |
|---|---|---|---|---|---|---|---|---|
| 2 | Resting | 72 | 72 | 140/85 | 16 | 100 | 10 | 8 |
| | Exercise | 84 | 84 | 165/100 | 16 | | | |
| 3 | Resting | 92 | 92 | 140/80 | 20 | | | |
| | Exercise | 122† | 132 | 160/90 | 26 | 120 | 14 | 9 |
| | | 112‡ | 112 | 160/85 | .. | | | |
| 4 | Resting | No conversion effected | | | | ... | .. | .. |
| | Exercise | | | | | | | |

* Return to resting levels ranged between one and two minutes.
† Six-inch step exercise.
‡ Four-inch step exercise (up and down to patient's fatigue level) 14 times.

then proceeded to quinidinize the patient. Because ECG coverage could not be obtained for the next two days, she was maintained on 0.4 gm. quinidine q. i. d.

The day after that she began to show signs of toxicity: nausea and vomiting, and the quinidine was stopped for that day. The following day she was again given 0.6 gm. q. 2 h. for five doses (plus belladonna to control the nausea and vomiting).

| | Quinidine, Gm. Q. 2 H. for 5 Doses |
|---|---|
| First day | 0.2 |
| Second day | 0.4 |
| Third day | 0.6 |

The same regimen was followed the next day, using 0.8 gm. of quinidine per dose. Conversion occurred during the night, but when maintenance on 0.8 gm. of quinidine q. i. d. was attempted fibrillation recurred almost immediately.

We then tried 1.0 gm. q. 2 h. for five doses (plus belladonna), but the heart remained in fibrillation, and we admitted failure to convert to regular sinus rhythm in this case.

## COMMENT

On the basis of the findings of other authors (as described), we have attempted to improve the efficiency of hemiplegic patients with auricular fibrillation and a history of embolization in carrying out programs prescribed for their rehabilitation.

Our method in general was to attempt conversion of auricular fibrillation to regular sinus rhythm in hemiplegics whose progress was slow or minimal. Four cases were used.

In our first case the episode of fibrillation was caught at its inception, and, since the cardiac reserve was markedly decreased, we attempted conversion with quinidine. This was successful, and the improvement was so marked that it stimulated our interest in this study. We then adopted the method of quinidine administration and exercise-tolerance tests as described.

In the next two cases, we studied the patient's hemodynamics and exercise tolerance both before and after conversion. There was amelioration of the cardiac reserve and improvement in the patients' ability to carry out the prescribed physical activity. There were no toxic reactions to the quinidine, and in these cases there was no embolic episode.

The last case showed only nausea and vomiting as toxic reactions to quinidine. These symptoms were controlled with belladonna. When there was no conversion to regular sinus rhythm on a dosage schedule of 0.1 gm. of quinidine q. 2 h. for five doses, we admitted failure to convert.

In the first case, conversion permitted the patient to undertake a program of ambulation, etc., which required a degree of physical effort that was impossible while her heart was fibrillating.

In the other two cases where conversion was effected, there was both subjective and objective improvement. Hemodynamics were improved, as were cardiac reserve and efficiency. In both cases there was a significant increase in the rate of progress on the program. This observation was based on clinical appraisal as well as on the judgment of the physical and occupational therapists who were in daily contact for hours with the patients. These therapists were not aware of the fact that these particular patients were being studied as outlined above.

In regard to the merits of conversion, a discussion of the various schools of thought on conversion of chronic fibrillation to regular sinus rhythm, or the difficulty or ease of conversion, would be beyond the scope of this paper. However, the method of quinidine administration is based on the quinidine blood level studies of M. Sokolow,† and our results are generally in agreement with his.

We are aware of the fact that our results are subjective in nature for the most part, but nevertheless the patients' improvement was significant, and we feel that further study is indicated.

## SUMMARY

In an effort to improve their ability to carry out the various physical activities of a program of rehabilitation, conversion of auricular fibrillation to regular sinus rhythm was attempted in four hemiplegic patients with a history of one or more embolizations.

† References 9 and 10.

In three of the four cases the heart rate was successfully converted to regular rhythm with an improvement in the patients' efficiency clinically and in their hemodynamics.

Further study is warranted.

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# A Bibliography of Internal Medicine

## MENINGOCOCCUS INFECTION

ARTHUR L. BLOOMFIELD, M.D., San Francisco

The rationale of this bibliography has been set forth in numerous other installments; suffice it to say that it is designed for the general physician rather than for the specialist.

As regards meningococcal infection, the situation is confused by the great variety of syndromes caused by the Meningococcus—epidemic meningitis, sporadic meningitis, chronic Meningococcus sepsis without meningitis, acute meningococcal bacteremia without meningitis—the unity of which was not suspected until isolation and identification of the organism was possible. We have found no comprehensive modern bibliography of the subject. The early literature is largely concerned with the descriptions of epidemics; it is thoroughly reviewed and documented by Hirsch (Ref. 8). Good general bibliographies are to be found in Foster and Gaskell's book (Ref. 25) and in the treatise of C. Worster-Drought and Alexander Mills Kennedy (Cerebro-Spinal Fever, London, A. & C. Black, Ltd., 1919).

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**1. Vieusseux.** Mémoire sur la maladie qui a regné à Genêve au printemps de 1805, *J. méd. chir. et pharm., Paris* **11:** 163, 1806.

The outbreak at Geneva in 1805 described by Vieusseux is generally taken as the starting point of modern study of epidemic meningitis. As here described, one was dealing with a new and violent disease of high mortality featured by sudden prostration or collapse, intense headache, vomiting, at times convulsions, rapid feeble pulse, and often death in a few hours. Most of those who survived several days recovered. There is no clear description of petechial spots during life or of stiff neck or opisthotonos. At autopsy one saw only a congestion and reddening of the meninges. Vieusseux regarded this disease as definite and distinct from other fevers and named it "malignant non-contagious cerebral fever." In one family four children were rapidly stricken and all died. Tartar emetic was thought to be the best remedy.

One would not be absolutely certain of the nature of this disease, however, were it not for the autopsy reports of Matthey (Matthey, A.: Recherches sur une maladie particulière qui a régné à Genêve en 1805, *J. méd. chir. et pharm., Paris* **11:** 243, 1806). "The vessels of the meninges were greatly injected. A gelatinous 'humor,' colored by blood was spread over the entire surface of the brain. Over the posterior part of the lobes of the brain and in the interior one saw a yellowish, puriform matter without manifest alteration of the cerebral tissue" (p. 246). "The nervous system seems strongly and directly attacked, for all the symptoms proclaim a brain lesion. One sees the bloody engorgement, the lymphatic and puriform effusions which explain the inefficiency of the methods which are tried to prevent or dissipate them" (p. 249).

2. **North, E.** A Treatise on a Malignant Epidemic Commonly Called Spotted Fever, New York, T. & J. Swords, 1811.

"It is between four and five years since a new and singular disease, which has obtained the name of Spotted Fever first appeared in Medfield, in Massachusetts" says North in his preface (p. vii). "Since this time it has prevailed as an epidemic and has proved a most tremendous plague in a considerable number of towns in this state, in Massachusetts, in Rhode-Island, in the state of New-York, in Vermont, and elsewhere sweeping off great numbers of inhabitants. No regular treatise, calculated to give the public an entire view of this disease, has hitherto been published" (p. vii).

"Whether the spotted fever may be considered as only a variety of cyanache maligna, or scarlatina, and be arranged under the order *phlegmasia* or *exanthemata;* or whether it ought to be regarded as only a variety of the malignant petechial fever of authors; or whether it is a new species of disease, to be added to the list of human calamities, is yet to be determined" (p. 6).

The disease frequently occurred in a very violent form; the descriptions resemble those of the bacteremic cases seen in World War I (Ref. 14). North mentions in his description "A great surprising sudden loss of strength," "violent pain of the head and many times of the limbs," "syncope," "coma," "mania," "delirium," and "petechiae" (pp. 10-12). "So frequent indeed was this species of hemorrhage during the first season in which the disease prevailed, that it was considered as one of its most striking characteristics, and gave rise to the name *petechial* or *spotted fever,* which has been very generally, though very improperly, applied to the disease. In size they [the spots] were various, commonly the head of a pin and a six cent bit would mark the two extremes" (p. 13). Under Section III (p. 15), "On the More Unusual Symptoms of the Fever," it is stated: "These are a dilatation, and, in some, a contraction of the pupils of the eyes, redness and suffusion of the eyes; blindness in some, in others double or treble vision; a drawing back of the head, with a kind of clonic spasm of the muscles of the neck." It seems clear that these cases which correspond more with the present-day sporadic type were relatively less common than the hyperacute (bacteremic?) form. As to the cause, North is straight 18th century: "The spotted fever is asthenic in its nature. Hence the remote and predisponent causes must be such as debilitate the system" (p. 25). There is no suggestion of contagion. The long section on treatment is hardly worthy of review; North leans heavily on Syndenham and on Huxham.

Indeed it would be difficult to be sure of the nature of this disease were there not appended autopsy notes of two cases by Drs. L. Danielson and E. Mann: "The second examination was made twelve hours after death, on the body of a girl of five years old, of the same family. Between the dura and the pia mater was effused a fluid resembling pus" (p. 95).

The last half of the book is of special interest; it consists of letters from physicians, copies of reports of cases, and other material dealing with the epidemic.

A great problem in the bibliography of cerebrospinal meningitis is the fact that for nearly a century the only recognized form

was the epidemic. Most of the outbreaks occurred in small towns, in garrisons, or in other odd places and were described by local doctors with inadequate documentation and usually in obscure or inaccessible medical journals. Two examples are "An Inquiry into the Nature and Treatment of the Prevailing Epidemic Called Spotted Fever, by Job Wilson, M.B., Boston, Bradford & Read, 1815," and "A History and Description of an Epidemic Fever Commonly Called Spotted Fever by E. Hale, Jr., M.D., Boston, Wells & Lilly, 1818." Little was added therefore to the knowledge of the disease until the discovery of the causal organism (Ref. 9). As to sporadic cases, one searches in vain through the older treatises for anything definite. Without bacteriological study and without lumbar puncture everything is in confusion, and whether the case was one of tuberculous meningitis, of pyogenic meningitis, or of Meningococcus meningitis can not be told. In Andral's great collection, for example (Andral, G.: Clinique médicale ou choix d'observations recueilles à l'hôpital de la charité, Paris, Fortier, Masson et Cie, 1840) one finds (Vol. 5, p. 65, 17th observation) a report of a case of "intense headache at start, with vomiting. Tendency to sleepiness and immobility. Gradual development of coma, more and more profound." At autopsy "purulent infiltration of the pia mater at the base of the brain and cerebellum." Whether this was Meningococcus meningitis one can not of course say.

The various epidemics are, however, listed in Hirsch (Ref. 8).

**3. Broussais, C.** Histoire des méningites cérébro-spinales, Paris, Moquet et Hanquelon, 1845.

During the years preceding this book there had been numerous and severe outbreaks of epidemic meningitis in troops in garrison all over France. The explanation of this distribution of the disease, now well understood (Ref. 26), was a cause of great perplexity to contemporary observers. At any rate, the army doctors rendered reports of various local outbreaks and Broussais abstracted and condensed them in his book, the special virtue of which is the numerous careful case reports with autopsy studies. Broussais clearly defined the disease. Aside from the excellent descriptions, however, there is little of value. Broussais missed his chance of making a fundamental epidemiological observation. "Do the migrations of the various regiments from one part of France to another account for the propagation of the epidemic?" he says (p. 14) and then adds "We shall see that it is impossible to accept this explanation."

**4. Niemeyer, F.** Die epidemische Cerebro-Spinal-Meningitis, Berlin, August Hirschwald, 1865.

Niemeyer's monograph was based mostly on personal observations of epidemics in Freiburg, Carlsruhe, and Rastat. Epidemic meningitis had long been thought, especially by the French, to be a variant of typhus, typhoid, or some sort of "spotted" fever. Niemeyer takes issue with this point of view. He insists that the important symptoms all pertain to intracranial disease, and that when convalescence is delayed "it depends in unmistakable fashion wholly on the residuals of the meningitis" (p. 9). This is all confirmed at autopsy, where the only significant change is "a widespread inflammation of the coverings of the brain and spinal cord" (p. 10). Furthermore, "the changes in the brain coverings in epidemic meningitis are exactly the same as those found in the sporadic form" (p. 10). There are no significant changes in the lymph glands or spleen. Niemeyer is however not sure that the disease is an infection, and he was willing to admit that atmospheric or telluric causes might be at fault, although he declined to rule out the probability of infection mainly on epidemiological grounds. He evidently saw no fulminating hyperacute cases such as those described by the Americans (Ref. 5).

This introductory chapter is followed by a detailed description of the pathological anatomy based on 15 cases. Under symptoms are listed severe headache, pains in the neck and back, stiffness of neck, pains in the extremities, hyperesthesia, tetanic cramps in

the neck and back muscles, convulsions, cranial nerve and other palsies, psychic disturbances, deafness, ptosis, diplopia, pupillary disturbances, inflammatory exudate in joints, fever, etc. He concludes this section with the following dicta: "All symptoms observed in the course of epidemic cerebrospinal meningitis are clearly accounted for by the purulent inflammation of the pia of the brain and cord" (p. 67). Sixty-nine and nine-tenths per cent of those affected died; some of the survivors were deaf or had visual disturbances.

"The treatment consisted mainly in the energetic use of cold in the form of ice packs to the head, leeches behind the ears and calomel internally" (p. 71).

The monograph of Mannkopff (Mannkopff, E.: Über Meningitis cerebro-spinalis epidemica, Braunschweig, Fredrich Viewig und Sohn, 1866) is noteworthy because of its excellent historical introduction; brilliant and detailed clinical account, including that of opisthotonus, and complete pathological description. There is then a long philosophical discussion of etiology and nature of the disease which leads to nothing definite.

**5. Stillé, A.** On the Proper Designation of the Present Epidemic (Cerebro-Spinal Meningitis), *Am. J. M. Sc.* **49:** 121, 1865.

The extensive recrudescence of epidemic meningitis during the preceding ten years had stimulated an immense amount of discussion as to the nature of the disease and how to classify it. The American journals are full of brief articles reporting a few cases, usually without autopsy. Stillé gives a critical discussion of the whole subject which throws important light on contemporary views. He points out that, although the disease was first named spotted fever, even in the early descriptions skin hemorrhages were by no means constantly present; he makes a plea for the designation cerebrospinal meningitis. He then points out the confusion which has arisen with typhus. This was evidently due to the occurrence of very acute cases (probably with bacteremia) in which, at autopsy, when the skull was opened bloody serum gushed out but no actual pus. In patients who lived longer, however, with a less fulminating disease, when the pia was opened frank pus was found. Stillé did useful work in tracing this spectrum. Lidell (Lidell, J. A.: On epidemic Cerebro-Spinal Meningitis or Spotted Fever, with Cases, *Am. J. M. Sc.* **49:** 17, 1865) reports patients who died in a day or two after onset. "While sawing off the calvarium and, subsequently removing the brain, a *large quantity of serum* coloured with blood flowed away." Draper (Draper, W. H.: Cerebro-Spinal Meningitis, or Spotted Fever, *Am. M. Times* **9:** 99, 111, 1864) describes cases he has seen "with terrible fatality at Carbondale, in the coal regions of Pennsylvania." "Many cases terminated within 12 hours." "In cases which are rapidly fatal, the lesion consists simply in an intense engorgement of the sinuses, veins, and minute vessels of the pia mater, with varying amounts of serous subarachnoid and ventricular effusion. In more protracted cases the arachnoid is lifted, and the surface of the brain obscured by a layer of consistent greenish pus. These inflammatory exudations are sometimes confined to the surface of the convolutions, sometimes to the base of the brain, and in many instances are found in both localities." The very high mortality, in some outbreaks nearly 100%, is stressed, and the futility of known therapy is emphasized. In the discussion on etiology Draper states "The question of contagion in this malady is one of great interest and importance. Most authorities agree that it is non-contagious. This, I believe, is the conclusion, without exception of all observers of the disease in this country."

Stillé also embodied his ideas in a book (Epidemic Meningitis, or Cerebro-Spinal Meningitis, Philadelphia, Lindsay & Blakiston, 1867).

**6. Strümpell, A.** Zur Pathologie und pathologischen Anatomie der epidemischen Cerebrospinal-meningitis, *Deutsch. Arch. klin. Med.* **30:**500, 1882.

Important clinical and pathological observations on epidemic meningitis. Strümpell quotes a finding of Professor Weigert of "an intensive purulent inflammation of the su-

perior naso-pharynx. This stimulated the thought that the inflammation in the naso-pharynx could furnish a starting point for the investigation of the manner in which the infectious material of epidemic meningitis enters the body especially the areas apparently so shut off as the brain and spinal cord." To this observation many later German writers trace the doctrine of the naso-pharynx as portal of entry for the Meningococcus.

**7. Kernig, W.** Über ein wenig bemerktes Meningitis Symptom, *Berlin klin. Wchnschr.* **21:** 820, 1884. [Original, Vrach, St. Petersburg, 1884, Vol. 5, pp. 427 and 446.]

Kernig describes his well known sign, emphasizing its occurrence in epidemic meningitis. With the leg flexed at the hip it is mechanically impossible to extend the knee (Beugestarre).

**8. Hirsch, A.** Handbuch der historisch-geographischen Pathologie, Ed. 2, Stuttgart, Ferdinand Enke, 1886, Vol. 3, p. 379. [English translation by Charles Creighton, London, New Sydenham Society, 1886, Vol. 3, p. 547.]

A detailed, extremely well-documented account of the innumerable epidemics of "meningitis cerebro-spinalis epidemica," which, first described in Geneva in 1805 (Ref. 1), occurred with increasing frequency through Europe and the United States. Even as late as 1886 the disease was thought of as essentially epidemic, and Hirsch makes no mention of sporadic cases as such. Hirsch noted that some epidemics were largely confined to children; he also pointed out the frequency of epidemics in soldiers in barracks, the explanation of which is now of course clear (Ref. 26); writing at the beginning of the bacteriological era, Hirsch questioned the claims of various bacteriologists (Ref. 9) and concluded that the virus was unknown and the disease "not contagious but transportable."

**9. Weichselbaum, A.** Über die Ätiologie der akuten Meningitis cerebro-spinalis, *Fortschr. Med.* **5:** 573, 1887.

The paper is a model of precise work. It was based on postmortem examination of six cases of cerebrospinal meningitis in which Weichselbaum found "an entirely different kind of bacteria" than the pneumococci and miscellaneous organisms which were recovered from most sporadic cases of meningitis. In the first case there were found in the meningitic exudate and in the ventricular fluid in cover-slip preparations a moderate number of cocci usually arranged in pairs with the side toward each other flattened so that each coccus represents a half sphere. "They lie either free between the pus cells or inside them where they are present in considerable numbers and remind one of gonococci." Similar organisms were seen in all the other five cases. Weichselbaum was able to cultivate them on agar; the growth was fairly delicate, and transfer through many generations was difficult. They appeared in smears of culture in pairs, tetrads, or small clumps. Some diplococci were larger than other cells. They were stained well with methylene blue and were decolorized by Gram's method. They were abundant in sections through the meninges. "Because of its characteristic form I will designate this variety of coccus as Diplococcus intracellularis meningitidis." Weichselbaum did not isolate the organism during life; that was first done by Heubner (Ref. 12). Injections of culture in rather large quantities killed mice, guinea-pigs, rabbits, and dogs with various lesions and organisms in the blood stream. Weichselbaum suggested the paranasal sinuses or the middle ear as the portal of entry in human cases.

Interestingly enough, Weichselbaum discussed his organisms simply as a cause of one group of meningitis cases; he says nothing about having discovered the cause of epidemic meningitis.

**10. Jaeger, H.** Die Transportmittel gewisser Infectionstoffe und Vorschläge zur Vernichtung derselben am Krankenbette, im Hausholt, im Verkehr, *Deutsche med. Wchnschr.* **20:** 409, 1894.

The German ministry of health as early as 1888 (Erlass des kgl. Ministers der 2 Cl. Medizinal-Angelegenheiten, die epidemische Genickstarre [Meningitis cerebrospinalis] betreffend, Veröffentl. d. kais. Gsndhtsamte **12:** 751, 1888) laid down rules for isolation and handling of cases of meningitis based on the conviction that the disease is "communicable and contagious." The disease was made reportable; the patients were to be isolated; the room and its contents, especially handkerchiefs, were to be cleaned and disinfected.

Jaeger reached the conclusion that the portal of entry for epidemic meningitis was from the nose through the lamina cribrosa to the meninges. He therefore examined nasal secretions and in four of five cases found "unequivocal" meningococci, and from contaminated hankerchiefs he grew the organism in pure culture. No meningococci were recovered from the secretions of eight healthy people.

This appears to be the first study of Meningococcus carriers, assuming, which seems to us very doubtful, that the organisms were meningococci and not Micrococcus catarrhalis.

11. **Jaeger, H.** Zur Ätiologie der Meningitis cerebrospinalis epidemica, *Ztschr. Hyg. u. Infectionskr.* **19:** 351, 1895.

Weichselbaum described his cocci in sporadic cases of meningitis which were difficult to distinguish from those due to various other organisms, especially Pneumococcus. He was not too emphatic in his claims, especially as he was unable to produce the typical disease in animals. Otto Leichtenstern (Über Meningitis cerebro-spinalis epidemica, *Deutsche med. Wchnschr.* **11:** 391; 537, 1885) had already found organisms, which seemed partly to meet Weichselbaum's description, in several cases of meningitis, and F. Goldschmidt (Zur Aetiologie der Meningitis cerebrospinalis, *Centralbl. Bakt.* **2:** 649, 1887) reported similar confirmatory findings. Nonetheless, the matter remained in dispute, and little more work was done for a number of years. Jaeger studied an outbreak of definite epidemic meningitis and isolated uniformly from the meninges of fatal cases an organism which he regarded as that of Weichselbaum. He also found it in sections of tissues and grew it in culture. As to animal inoculation, he found as did Weichselbaum that subcutaneous injection was totally ineffective. He concluded that the Meningococcus was a different organism from the Pneumococcus, of which not every one had as yet been convinced, and he definitely insisted that the organism of Weichselbaum is the significant finding in epidemic meningitis.

However, there were several odd points in the findings of Jaeger. The organisms did not always decolorize with the Gram stain; they sometimes occurred in chains, and they could be isolated from dried nasal secretions. What was Jaeger actually dealing with? At any rate, it remained for H. Albrecht and A. Ghon (Über die Aetiologie und pathologische Anatomie der Meningitis cerebrospinalis epidemica, *Wien. klin. Wchnschr.* **14:** 984, 1901) to give a complete and critical analysis of the literature to date as well as observations of their own and to settle definitely that the cause of epidemic meningitis was the organism of Weichselbaum, to explain that it was always Gram-negative, always fragile and difficult to cultivate, and essentially nonpathogenic for small animals. The findings reported by others of Gram-positive forms, or organisms resembling pneumococci, of readily cultivable organisms were clearly either contaminations or mixed infections. This paper is definitive and settled for all time a much vexed question. Albrecht and Ghon were also among the first to definitely isolate true meningococci from the nasopharynx.

12. **Heubner, O.** Beobachtungen und Versuche über den Meningococcus intracellularis (Weichselbaum-Jaeger), *Jahrb. Kinderheilk.* **43:** 1, 1896.

Heubner was the first, by means of lumbar puncture, to recover Meningococcus from the spinal fluid during life. He found typical "biscuit shaped" diplococci in pus cells; they are also described as like a pair of coffee beans

side to side. He injected pus containing meningococci intraspinally into goats and produced definite meningitis. He grew the organism in pure culture. He emphasized the value of spinal puncture in clinical practice in differentiating various forms of meningitis, although others such as Lichtheim (Zur Diagnose der Meningitis, *Berlin klin. Wchnschr.* **32:** 269, 1895) had already dealt with the general subject following Quincke's introduction of lumbar puncture and detailed description of the technique (Quincke, H.: Über Hydrocephalus, Verhandlungen des Congressus für innere Medizin, Vol 10, Wiesbaden, J. F. Bergman, 1891, p. 321).

**13. Councilman, W. T.; Mallory, F. B., and Wright, J. H.** Epidemic Cerebro-Spinal Meningitis and Its Relation to Other Forms of Meningitis: A Report of the State Board of Health of Massachusetts, Boston, Wright & Potter, 1898.

A comprehensive summary of all phases of the disease supplemented by the authors' own early observations in which they confirmed the work of Weichselbaum (Ref. 9). There is an excellent historical section.

**14. Osler, W.** The Arthritis of Cerebro-Spinal Fever, *Boston M. & S. J.* **139:** 641, 1898.

In the course of a report of a case of epidemic meningitis with inflammation of the joints and other evidence of sepsis Osler states that Meningococcus was isolated from the blood during life. N. B. Gwyn (A Case of General Infection by the Diplococcus Intracellularis of Weichselbaum, *Bull. Johns Hopkins Hosp.* **10:** 112, 1899) reported on the bacteriology of the patient. Two blood cultures were positive for Meningococcus. "This is believed to be the first instance recorded in which general infection, or septicemia has been demonstrated in this disease." H. Cochez and Lemaire (Relation de l'épidémie de méningite cérébrospinale à Alger et dans les environs, *Arch. gén. méd.* **189:** 574, 1902) were able to grow meningococci from the blood of two patients with meningitis who recovered.

H. Salomon (Über Meningococcusseptikämie, *Berlin klin. Wchnschr.* **39:** 1045, 1902) describes a patient who obviously had a septic fever with purpura (spotted fever) from whose blood Meningococcus was recovered. Irregular septic fever continued, however, for several months, with repeated isolation of Meningococcus from the blood. Eventually the patient developed signs of meningitis but recovered. "For two months the meningococcus circulated in the blood while we waited from day to day for meningitis to appear. But finally it found its accustomed localization in the intracranial space." This, of course, is a classical case of chronic Meningococcus sepsis. However, meningococci were soon isolated from the blood in regular cases of meningitis, as, for example, that of A. Marcovich (Meningococcen im kreisenden Blute, *Wien. klin. Wchnschr.* **19:** 1312, 1906), who reviews the early literature.

Meanwhile F. W. Andrewes (A Case of Acute Meningococcal Septicemia, *Lancet* **1:** 1172, 1906) described a patient in collapse, covered with purpura, from whose blood meningococci were grown and in whose blood meningococci were seen intra vitam within the leucocytes. At autopsy there were hemorrhages everywhere and "Both suprarenals were in an intensely hemorrhagic condition, dark purple in color, swollen, and contrasting strongly with the kidneys which were but slightly congested"; the case was obviously one of the Waterhouse-Friderichsen syndrome.*

Elser and Huntoon (Studies on Meningitis, *J. M. Res.* **20:**377, 1909) isolated meningococci from the blood in 11 of 41 cases of meningitis. They thought that the organism

* Thus Andrewes antedated Waterhouse's case by five years (Waterhouse, R.: A Case of Suprarenal Apoplexy, *Lancet* **1:**577, 1911). It is of interest that in the Report of the Pathologist in the Middlesex Hospital Reports for 1894, there is a pathological report (p. 279) of a case with profuse purpura and bilateral hemorrhages into the suprarenal capsules. The modern literature is reviewed by L. D. Weinstein and T. H. McGavack (The Waterhouse-Friderichsen Syndrome, *New England J. Med.* **232:**95, 1945).

first gains access to the blood and secondarily localizes in the meninges. The idea of a preliminary bacteremic stage of epidemic meningitis was strongly emphasized by W. W. Herrick (The Intravenous Serum Treatment of Epidemic Cerebrospinal Meningitis, *Arch. Int. Med.* **21:** 541, 1918). He recognized, in an epidemic in an army camp, "primary meningococcus sepsis in almost half of the cases before the characteristic selective action on the meninges has been exerted," and advocated brisk use of serum intravenously as well as intraspinally.

Thus all types of Meningococcus infection are illustrated, and with any there may be a bacteremia.

**15. Goodwin, M. E., and von Sholly, A. L.** The Frequent Occurrence of Meningococci in the Nasal Cavities of Meningitis Patients and of Those in Direct Contact with Them. *J. Infect. Dis.*, Supp. 2, p. 21, 1906.

The writers review the earlier papers in which meningococci were claimed to have been isolated from the nasal sinuses or nasopharynx. They conclude that the cultures of only 14 cases in the literature were studied with sufficient care to warrant their acceptance as true meningococci, and they point out that meningococci are easily confused with other Gram-negative organisms of the catarrhalis group. A. Ghon and H. Pfeiffer (Der Micrococcus catarrhalis (R. Pfeiffer) als Krankheitserreger, *Ztschr. klin. Med.* **44:** 262, 1902) give a detailed discussion of this group, point out their frequent occurrence in the upper air passages, and discuss the differentiation of them from gonococci and meningococci. They emphasize the coarse opaque colonies with ragged edges and the spontaneous agglutinability. Although they were not clear that catarrhalis is a member of the normal basic flora of the upper air passages, they assigned it a very subordinate role in disease. H. Jaeger (Die specifische Agglutination der Meningococcen als Hilfsmittel in ihrer Artbestimmung und zur bacteriologischen Diagnose der epidemischen Genickstarre, *Ztschr. Hyg. u. Infectionskr.* **44:** 223, 1903) prepared immune sera in rabbits with meningococci, and with these was able to recognize specifically other strains and in general emphasized the value of the procedure. M. catarrhalis uniformly failed to agglutinate in these sera. Goodwin and von Sholly made nasal cultures with the greatest care. Meningococci were isolated from 17 to 37 patients with meningitis when culture was made during the first two weeks of the disease, whereas in 5 of 45 healthy persons living in close contact with meningitis patients meningococci were also isolated. The organisms were identified by careful morphological and cultural criteria and by agglutination tests. They conclude "Meningococci were isolated from nasal mucus of 50 per cent of meningitis patients and from about 10 per cent of the people in closest contact with them. They were frequently present in enormous numbers. The finding of meningococci in great numbers in the nasal mucus of such a large proportion of the patients and of those caring for them, and the absence of meningococci from the nasal mucus of a large number of normal persons examined would strongly indicate the necessity of isolating cases of cerebro-spinal meningitis," a conclusion reached by the Germans as early as 1888 (Ref. 10).

**16. Kutscher, K.** Über Untersuchungen der Nasenrachenhöhle gesunder Menschen auf Meningococcen. *Deutsche med. Wchnschr.* **32:** 1071, 1906.

It was already common knowledge that meningococci are frequently found in the nasopharynx of patients with meningitis and of contacts. Kutscher made cultures from healthy people who had no known contact with meningitis patients at a time when no clinical cases existed. In 56 people he isolated meningococci four times; all were agglutinated by specific sera in a titer of 1 : 500 to 1 : 1000. The source of these organisms and the epidemiological implications are discussed. Similar findings have since been obtained innumerable times by others.

**17. Jochmann, G.** Versuche zur Serodiagnostik und Serotherapie der epidemischen Genickstarre, *Deutsche med. Wchnschr.* **32:** 788, 1906.

It occurred to Jochmann to try to prepare a potent anti-Meningococcus serum as a therapeutic agent and also for diagnostic purposes, especially to separate true meningococci from other Gram-negative cocci. He immunized a horse first with dead and then with living culture; the serum attained a titer of 1:1500. The serum specifically agglutinated true meningococci, as Jaeger found (Ref. 11). Jochmann soon discovered that a considerable number of healthy people in the vicinity of an epidemic harbored meningococci in the nasopharynx, as had Goodwin and von Sholly (Ref. 15). M. catarrhalis was not agglutinated. Jochmann laid the ghost of widespread claims that there were Gram-positive varieties of meningococci by showing that specifically agglutinable strains were always Gram-negative. He also stressed the fact that his serum was polyvalent. The serum protected mice and guinea pigs against intraperitoneal injection of meningococci. Jochmann then treated some 40 human cases, first with subcutaneous injection and later with intraspinal serum. Some of the patients seemed to be helped, but Jochmann concludes with the greatest conservatism that at least the serum did no harm. These observations opened the long era of serotherapy of epidemic meningitis, which did not end until about 1940. Kolle and Wassermann (Versuche zur Gewinning und Wertbestimmung eines Meningococcenserum, *Deutsche med. Wchnschr.* **32:** 609, 1906) also did important early work on preparing an immune serum.

**18. Flexner, S.** Concerning a Serum Therapy for Experimental Infection with Diplococcus Intracellularis, *J. Exper. Med.* **9:** 168, 1907.

Flexner's interest in meningitis began in connection with the New York epidemic of 1904-1905. His first paper (Flexner, S.: Contributions to the Biology of Diplococcus Intracellularis, *J. Exper. Med.* **9:** 105, 1907) dealt with the cultural and other biological characteristics of Meningococcus. Although Annibal Bettencourt and Carlos França (Über die Meningitis cerebrospinalis epidemica und ihren specifischen Erreger, *Ztschr. Hyg. u. Infectionsk.* **46:** 463, 1904) had failed to produce experimental meningitis in various animals, including monkeys, Flexner (Experimental Meningitis in Monkeys, *J. Exper. Med.* **9:** 142, 1907) attempted again to produce it by introducing culture material by lumbar puncture. He used huge quantities of bacteria. The animals usually died in a few hours or recovered promptly. In the fatal cases "meningitis" was found, but the organisms tended to die out quickly, and it is doubtful if Flexner produced a condition really analogous to the human disease. From this point he went on to treat the experimental disease with immune serum. "In summarizing these experiments, it may be said that by employment of an homologous antidiplococcus serum several monkeys were saved from death due to experimental infection with Diplococcus intracellularis. The monkeys could, by simultaneous injection of serum and culture be prevented from developing severe symptoms." However, Flexner found "a certain definite protective value" in normal monkey serum also. Flexner suggested the possibility of developing "more active antisera" for use in human meningitis.

A preliminary report of all the above work appeared in August, 1926 (Flexner, S.: Experimental Cerebrospinal Meningitis and Its Serum Treatment, *J. A. M. A.* **47:** 560, 1906).

**19. Flügge [C.].** Die Verbreitungsweise und Bekämpfung der epidemische Genickstarre, *München med. Wchnschr.* **54:** 1059, 1907.

Flügge summarizes concisely the situation as regards distribution of meningococci in relation to spread of the disease. He states that all patients with epidemic meningitis harbor meningococci in the nasopharynx for the first five days, after which they usually disappear rapidly. Among contacts about 70% are found to be carriers for three weeks or so. Since the Meningococcus is a delicate organism, which does not survive well outside the body, the disease must be spread by

carriers. Flügge had already become interested in meningitis during the German epidemic of 1905 (Die im Hygienischen Institut der Königl. Universität Breslau wahrend der Genickstarre-Epidemie im Jahre 1905 Ausgefürten Untersuchungen, *Klin. Jahrb.* **15:** 353, 1906). This volume also contains many articles by German authorities such as Kolle and Wassermann, v. Lingelsheim, and others which are a mine of information on the details of the disease; they are all based on the material studied in this great epidemic.

20. **Liebermeister, G.** Über Meningococcensepsis, *München med. Wchnschr.* **55:** 1978, 1908.

Liebermeister seems to have been the first to recognize chronic Meningococcus sepsis as a definite clinical entity apart from epidemic meningitis. He reports a classical case with arthralgia, prolonged irregular fever, positive blood cultures, but normal spinal fluid, with recovery. The voluminous literature on this syndrome is comprehensively reviewed by Dopter (Ref. 27, p. 136 et seq.). Poorly documented cases are however found in earlier reports, such as those of E. Sacquépée and E. Peltier (Méningites cérébrospinales grippales, *Arch. gén. méd.* **187:** 537, 1901) and of A. Cochez and Lemaire (Ref. 14). Dock in 1924 (Dock, W.: Intermittent Fever of 7 Months Duration Due to Meningococcemia, *J. A. M. A.* **83:** 31, 1924) was able to collect 68 cases from the literature.

21. **Flexner, S., and Jobling, J. W.** Serum Treatment of Epidemic Cerebro-Spinal Meningitis, *J. Exper. Med.* **10:** 141, 1908.

The writers immunized a horse with a mixture of Meningococcus strains, first given subcutaneously and later intravenously. The titer of the serum is not stated. Forty-seven cases of epidemic meningitis were treated by one to three intraspinal injections of the serum. Seventy-two and three-tenths per cent of the patients recovered, whereas of untreated cases in the same epidemic about 70% died. Flexner and Jobling are conservative but hopeful in their conclusions. A little later they reported (An Analysis of 400 Cases of Epidemic Meningitis Treated with the Anti-Meningitis Serum, *J. Exper. Med.* **10:** 690, 1908) a large number of cases with a detailed tabulation of results. Of these cases only 11% to 50% died, depending upon age, as against an over-all mortality of at least 75% in untreated cases. They conclude: "The antimeningitis serum when used by the subdural method of injection, in suitable doses and at proper intervals, is capable of reducing the period of illness; of preventing, in large measure, the chronic lesions and types of the infection; of bringing about complete restoration to health in all but a very small number of the recovered, thus lessening the serious deformity and permanent consequences of meningitis; and of greatly diminishing fatalities due to the disease." Meanwhile, Harvey Cushing and Frank J. Sladen (Obstructive Hydrocephalus Following Cerebrospinal Meningitis, Intraventricular Injection of Antimeningitis Serum (Flexner), *J. Exper. Med.* **10:** 548, 1908) called attention to the possible introduction of serum directly into the ventricles in cases with hydrocephalus and block.

But the Germans had not been idle. A. Wassermann (Über die bisherigen Erfahrungen mit dem Meningococcen-Heilserum bei Genickstarrekranken, *Deutsche med. Wchnschr.* **33:** 1585, 1907) reported several cases treated by subcutaneous and intraspinal injections of Jochman's serum with promising results, and C. Schöne (Überblick über die Behandlung von 30 Genickstarrkranken mit Jochman'schen Meningococcenserum, *Therap. Gegenwart.* **48:** 52, 1907) reported on 30 treated cases. They achieved results comparable with those of Flexner after intraspinal injection.

Flexner later (The Results of the Serum Treatment in 1300 Cases of Epidemic Meningitis, *J. Exper. Med.* **17:** 553, 1913) gave a comprehensive summary of the results of treatment with his serum of cases all over the world. The mortality of all treated patients was 30.9%; of those treated on the first to third day only 18.1% died. At the same time the mortality of untreated cases varied from

70% to 90%. Flexner discusses at length the difficulties and complications of serum therapy.

With World War I there developed numerous and intense epidemics of meningitis, especially in the armed forces. These epidemics stimulated not only the studies on classification (Ref. 24) but standardization of therapeutic sera, taking into account the different strains of Meningococcus. H. L. Amoss and M. Wollstein (A Method for the Rapid Preparation of Anti-Meningitis Serum, *J. Exper. Med.* **23:** 403, 1916) led the way in the preparation of potent polyvalent sera with a further report (Amoss, H. L., and Marsh, P.: Standardization of Antimeningitis Serum, *J. Exper. Med.* **28:**779, 1918) dealing largely with criteria for standardization. Serum therapy subsequently had a checkered career. In the late 1920's and 1930's there were severe outbreaks of meningitis which appeared refractory to available sera and had a mortality rate of 70% to 80%. The complexities of this situation in relation to classification of strains is reviewed by Branham (Ref. 24). It is interesting to note the way in which things have come full circle, so that in 1940 there were again emphasized only two groups of meningococci, Group I responsible for most of the recent epidemics and Group II responsible for most endemic cases and carriers.

**22. Dopter, C.** Étude de quelques germes isolés du rhinopharynx voisins du méningocoque (paraméningococcus), *Compt. rend. Soc. biol.* **67:** 74, 1909.

It did not occur to the early writers that there were different types of meningococci, distinguishable serologically. Dopter isolated from the nasopharynx organisms which were morphologically and culturally indistinguishable from meningococci and which gave the same reactions in sugars but which differed in certain serological respects. These organisms were to be differentiated from the pseudomeningococci of von Lingelsheim, which gave different reactions in sugars from true meningococci. Dopter felt that his parameningococci had, however, the same clinical significance as true meningococci. Dopter and Pauron (Différenciation des paraméningocoques entre eux par le saturation des agglutinins, *Compt. rend. Soc. biol.* **77:** 231, 1914) later extended these studies and differentiated various types of parameningococci. Up to this time, however, little or no attention had been paid to differences in strains in clinical work, and the sera of Flexner and of Jochmann were prepared from various meningococci, selected in empirical and haphazard fashion. Dopter led the way in purposeful and intelligent classification of Meningococcus strains.

**23. Netter, A., and Debré, R.** La méningite cérebrospinale, Paris, Masson et Cie, 1911.

This classical monograph fully summarizes knowledge of every phase of Meningococcus meningitis to date. The clinical descriptions are comprehensive and admirable and include complications such as paralysis and visual and auditory troubles. The chapter on the nasopharynx as habitat of the Meningococcus is an especially valuable summary. The authors offer an explanation for the widely varying number of carriers found by different observers, and they conclude that the intensity of the outbreak of meningitis and the carrier rate correspond. This was well shown later by Glover (Ref. 26).

**24. Gordon, M. H., and Murray, E. G.** Identification of the Meningococcus, *J. Roy. Army M. Corps* **24:** 411, 1915.

It was thought at first that all meningococci were identical. From about 1905 on, however, numerous workers began to distinguish various races or strains. The differentiation was made partly by morphological study, partly by fermentation of sugars, and partly by serological tests. Some of the early papers of importance are those of J. A. Arkwright (On Variations of the Meningococcus and Its Differentiation from Other Cocci Occurring in the Cerebro-Spinal Fluid, *J. Hyg.* **7:** 193, 1907; Varieties of the Meningococcus with Special Reference to a Comparison of Strains from Epidemic and Sporadic Sources, *J. Hyg.* **9:**104, 1909); K. Kutscher (Über Untersuchungen der Nasen-

rachenhöle gesunder Menschen auf Meningococcen, *Deutsche med. Wchnschr.* **32:** 1071, 1906); William J. Elser and Frank M. Huntoon (Studies on Meningitis, *J. M. Res.* **20:** 377, 1909), and Dopter (Ref. 22). None of these students, however, arrived at any definitive or uniform classification of meningococci. Arthur W. M. Ellis (A Classification of Meningococci Based on Group Agglutination Obtained with Monovalent Immune Rabbit Serums, *Brit. M. J.* **2:** 881, 1915) was able to divide meningococci into two groups by agglutination tests, and J. A. Arkwright (Grouping of Strains of Meningococcus Isolated During the Epidemic of Cerebrospinal Meningitis in 1915, *Brit. M. J.* **2:** 885, 1915) came to the same conclusion. His two main groups corresponded to Dopter's meningococci and parameningococci (Ref. 22).

Gordon and Murray, using improved methods for agglutination and absorption tests, were able to distinguish four definite serological types of meningococci among patients in the army. Further reports by Gordon appeared (Identification of Meningococcus, *J. Hyg.* **17:** 290, 1918; Cerebrospinal Fever, Medical Research Council, Special Report Series, No. 50, London, His Majesty's Stationery Office, 1920). This work had important implications in serum therapy and in epidemiological studies. Meanwhile, similar studies were being made in France by M. Nicolle, E. Debains, and C. Jouan (Études sur les meningocoques et les sérums antimeningococciques, *Ann. Inst. Pasteur* **32:** 150, 1918). While strains of meningococci have been found which do not fit Gordon's types, he brought order into the whole subject and his results have been of practical use. For recent comprehensive discussion of the Meningococcus see Sara E. Branham (The Meningococcus [Neisseria Intracellularis], *Bact. Rev.* **4:** 59, 1940; The Significance of Serological Types Among Meningococci, *J. A. M. A.* **108:** 692, 1937).

**25. Foster, M. and Gaskell, J. F.** Cerebro-Spinal Fever, London, Cambridge University Press, 1916.

An excellent survey to date of meningitis based largely on recent military experience. The historical section is especially valuable.

**26. Glover, J. A.** Observations of the Meningococcus Carrier Rates, and Their Application to the Prevention of Cerebrospinal Fever, in Cerebrospinal Fever, Medical Research Council, Special Reports Series, No. 50, London, His Majesty's Stationery Office, 1920, p. 133.

Glover carefully followed the carrier rate in a military installation where there was great crowding. Under these conditions with beds only 6 in. apart the carrier rate was found to rise from the normal of 2% to 4% to the amazing figure of over 70%, following which clinical meningitis broke out. When the beds were spaced 3 ft. apart the outbreak promptly subsided and the carrier rate fell to a low level. This phenomenon was repeated several times during periods of crowding. Here was final proof that carriers were the source of meningitis and that danger of clinical infection varied with the density of the healthy carrier population. A review of this subject is that of K. F. Maxey (The Relationship of Meningococcus Carriers to the Incidence of Cerebrospinal Fever, *Am. J. M. Sc.* **193:** 438, 1937). Of special interest are the studies of Geoffrey Rake (Studies on Meningococcus Infection: VI. The Carrier Problem, *J. Exper. Med.* **59:** 553, 1934). Rake followed carefully a small group of carriers and found that they fell into three categories, namely, chronic, intermittent, and transient carriers.

**27. Dopter, C.** L'Infection méningococcique, Paris, J.-B. Baillière et Fils, 1921.

This comprehensive work deals, like that of Netter and Debré (Ref. 23), with every phase of the subject and leaves little to be added today.

**28. Blackfan, K. O.** The Treatment of Meningococcus Meningitis, *Medicine* **1:** 139, 1922.

Blackfan reviews the whole history of treatment of Meningococcus Meningitis, including serum therapy.

**29. Banks, H. S.** Chemotherapy of Meningococcal Meningitis, *Lancet* **2:** 92, 1939.

The advent of the sulfonamides sounded the death knell of serum therapy in epidemic meningitis. Banks reported 31 cases treated with sulfanilamide without a death and 36 cases treated with M+B 693 with 1 death. On the whole, patients improved more rapidly with chemotherapy than with serum and the doing away with intraspinal therapy was a great advantage. "No evidence in favour of auxiliary treatment with serum was obtained." By 1943 Paul B. Beeson and Ethel Westerman (Cerebrospinal Fever, *Brit. M. J.* **1:** 497, 1943) were able to discuss sulfonamide therapy in 3575 cases, with an over-all fatality rate of 15.94%. Serum was also used in some cases; among 2591 cases in whom sulfonamides were used alone the fatality rate was 14.3%. Among patients from 15 to 25 years old only 5% to 8% died. Similarly, A. A. Jubb (Chemotherapy and Serotherapy in Cerebrospinal [Meningococcal] Meningitis, *Brit. M. J.* **1:** 501, 1943) found a death rate of 9.2% in 2357 cases treated by chemotherapy alone.

An earlier note is that of F. F. Schwentker, S. Gilman, and P. H. Long (The Treatment of Meningococcic Meningitis with Sulfanilamide, *J. A. M. A.* **108:** 1407, 1937), who treated 11 patients with sulfanilamide subcutaneously and intraspinally, with a mortality of 9%.

**30. Meehan, J. F., and Merrilees, C. R.** An Outbreak of Cerebrospinal Meningitis in a Foundling Hospital: The Treatment of Carriers with "M. and B. 693," *M. J. Australia* **2:** 84, 1940.

It was known that outbreaks of meningitis ran parallel to the carrier rate, but no simple secure method of extirpating meningococci from the nasopharynx had been discovered. The writers treated carrier children after an outbreak of meningitis in a foundling hospital with "B. and M. 693" [a sulfonamide] and immediately reduced the carriers from 75 to 3. Similarly, R. W. Fairbrother (Cerebrospinal Meningitis: The Use of Sulphonamide Derivatives in Prophylaxis, *Brit. M. J.* **2:** 859, 1940), in a very careful study, found that "adequate dosage with sulphapyridine is a satisfactory method of clearing meningococci from the nasopharyngeal mucosa." Finally, F. S. Chaver, B. B. Breese, and H. C. Upham (The Treatment of Meningococcus Carriers with Sulfadiazine, *Ann. Int. Med.* **19:** 602, 1943) found that "sulfadiazine is effective, since all of 161 meningococcus carriers receiving 8 grams of the drug over a period of 72 hours yielded negative cultures on the fourth day."

**31. Miller, C. P., and Foster, A. Z.** Studies on the Action of Penicillin: II. Therapeutic Action of Penicillin on Experimental Meningococcal Infection in Mice, *Proc. Soc. Exper. Biol. & Med.* **56:** 166, 1944.

The writers studied the effect of penicillin on experimental Meningococcus peritonitis in mice. There was a definite curative effect. Similarly (III. Bactericidal Action of Penicillin on Meningococcus in Vitro, *Proc. Soc. Exper. Biol. & Med.* **56:** 205, 1944) they found that "meningococci are readily killed by penicillin in nutrient broth at 37°."

**32. Rosenberg, D. H., and Arling, P. A.** Penicillin in the Treatment of Meningitis, *J. A. M. A.* **125:** 1011, 1944.

The writers reported the first sizeable series of meningitis cases treated with penicillin. They gave an intrathecal injection of 10,000 units every day as well as small doses intramuscularly. Sixty-four of sixty-five patients recovered. Finland and his associates also in an early report (Meads, M.; Hams, W. H.; Samper, B. A., and Finland, M.: Treatment of Meningococcal Meningitis with Penicillin, *New England J. Med.* **231:** 509, 1944) concluded that penicillin was less satisfactory than sulfonamides and acted more slowly. However, he had available only small doses of penicillin. Recently, Lepper and his associates (Lepper, H. H.; Dowling, H. F.; Wehrle, P. F.; Blatt, N. H.; Spies, H. W., and Brown, M.: Meningococcic Meningitis: Treatment with Large Doses of Penicillin Compared to Treatment with Gantrisin, *J. Lab. & Clin. Med.* **40:** 891, 1952) treated

Meningococcus meningitis with injections of 1,000,000 units of penicillin intramuscularly or intravenously every two hours without intrathecal injections. Of 40 patients only 1 died. These writers conclude that penicillin is just as effective as sulfonamides when large doses are used. The earlier studies (Dernoff-Stanley, E. D.; Dowling, H. F., and Sweet, L. K.: The Absorption into and Distribution of Penicillin in the Cerebrospinal Fluid, *J. Clin. Invest.* **25:** 87, 1946) which showed little if any penicillin in the spinal fluid after parenteral injections were done, as it turned out, with too small doses of the antibiotic. On the other hand, Schwenlen and his associates (Schwenlen, G. T.; Barton, R. L.; Bauer, T. J.; Loewe, L.; Bundeson, H. N., and Craig, R. M.: Penicillin in Spinal Fluid After Intravenous Administration, *J. A. M. A.* **130:** 340, 1946) found, working with syphilitics, that significant amounts of penicillin were demonstrated in the spinal fluid after intravenous drip of 10,000,000 to 25,000,000 units of penicillin in 24 hours.

# Central Nervous System Complications of Primary Atypical Pneumonia

LOUIS YESNICK, M.D., CHICAGO

Primary atypical pneumonia is characterized by a low mortality and relatively few complications.[1] Of these complications, those involving the central nervous system seem to be particularly infrequent. Except for the brief summary of Scarlini[2] (in Italian) no review on this specific aspect of atypical pneumonia has appeared in the literature. It is the purpose of this paper to review the reported cases and to add a case report.

## REPORT OF A CASE

A 34-year-old housewife was admitted to the Edgewater Hospital on Feb. 13, 1955. She presented a 10-day history of chill, fever, cough, and expectoration. During this time she had received penicillin, erythromycin U. S. P. (Ilotycin), and "sulfa," without any apparent improvement. Physical examination upon admission revealed a temperature of 101 F, pulse rate of 122, and respiratory rate of 24. Diminished breath sounds and rales were present over the left upper lobe.

A chest plate showed a left upper lobe infiltrate. The blood studies showed a RBC count of 4,780,000; Hb., 11.5 gm., and WBC, 14,950 (65% polymorphonuclear cells). Sputum samples were negative for acid-fast bacilli. Sputum culture showed a viridans Streptococcus as the predominant organism. Serum cold agglutinins were present in a titer of 1:80 for Type O cells and in a titer of 1:1280 for the patient's own red blood cells. Kahn test was negative. Coombs test was negative.

The patient was treated with tetracycline (Achromycin) hydrochloride U. S. P., 250 mg. orally q. i. d., and the usual supportive measures (fluids, acetylsalicylic acid, cough medicine, bed rest). She gradually improved and became afebrile on the morning of the fourth hospital day. A chest plate at this time showed considerable resolution of the left upper lobe infiltrate.

On the afternoon of the fourth hospital day she quickly became irritable, semioriented, restless, and confused. Her vision seemed impaired. The following morning she became incontinent of urine and feces. There was no neck rigidity. Transient Hoffman and Babinski signs could be elicited on the fifth hospital day. There was moderate bilateral papilledema. A spinal tap on the fifth hospital day revealed a pressure of 230 mm. of spinal fluid, which contained 44 mg. per 100 cc. of protein, 63 mg. per 100 cc. of sugar, and 81 cells per cubic millimeter (85% lymphocytes). Culture of the fluid revealed no growth. The colloidal gold curve was flat, and the Wassermann test was negative.

The patient remained in this confused, somnolent, irritable state for four days. On the evening of her seventh hospital day she rapidly improved. She recognized people, asked about her children, and was able to feed herself. The only manifest residual abnormality was euphoria and a persistent uninhibited demand for sexual gratification. This euphoria persisted for about two weeks and then gradually subsided. During the entire course of this central nervous system involvement she manifested no fever. She was discharged from the hospital two weeks after admission.

Blood was sent to the Illinois State Health Laboratory 30 days and 44 days after the onset of her illness. Negative complement-fixation tests were reported for Eastern and Western equine encephalitis, St. Louis encephalitis, lymphocytic choriomeningitis, mumps, psittacosis, and lymphogranuloma venereum.

## MATERIAL REVIEWED

Reimann[3] introduced the term "atypical pneumonia" in 1938. For this reason all case reports since 1938 were reviewed. Table 1 lists some pertinent data from these reports. Some of these were included reluctantly. Thus, the case report of Suchett-Kaye[4] appears to be one of bacterial pneumonia. The case reported by Ingleby[5] is complicated by the presence of bacterial

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From the Department of Medicine, the Chicago Medical School.

TABLE 1.—*Summary of Reported Cases*

| Case No. | Author | Age, Yr. | Sex | Complication | Day of Onset | C. S. F. Pressure, Mm. | C. S. F. Cells | C. S. F. Protein, Mg./100 Cc. | Course |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Reimann [8] | 35 | F | Meningo-encephalitis | 42nd | 220 | 350 (78% P*) | 74 | Died 55th day of illness |
| 2 | Perrone & Wright [19] | 20 | M | Meningo-encephalitis | 8th | ? | 76 (68% L*) | ? | Died 14th day of illness |
| 3 | Green & Eldrige [23] | 19 | M | Meningo-encephalitis | 14th | N* | 16 (P) | Inc. | Recovered in 4 days |
| 4 | Sheppe and others [24] | 37 | M | Ascending paralysis | 12th | 250 | 221 (87% L) | 40 | Recovered in 6 days |
| 5 | Campbell and others [13] | 24 | M M | Ascending paralysis | 9th | Inc. | 2000 (90% P) | Inc. | Died 12 hr. after onset of myelitis |
| 6 | Owen [25] | ? | M | Ascending paralysis (Guillain-Barré-like syndrome) | 6th | N | N | 326 | Recovered in 2 wk. |
| 7 | Gundersen [26] | Child | | Meningitis | ? | ? | Inc. | ? | "Improved rapidly" |
| 8 | Gundersen [26] | 38 | M | ? | 7th | Not done | | | Died 15th day of illness |
| 9 | Dingle [27] | ? | | Myelo-encephalitis | 18th | Acute hemmorhagic encephalitis on postmortem examination | | | |
| 10 | Baker & Noran [34] | 10 | F | Coma, right hemiparesis | 6th | ? | 17 (90% L) | ? | Recovered; ataxia and mental deterioration present 3 mo. later |
| 11 | Baker & Noran [34] | 1 mo. | F | Lethargy, somnolence | 10th | Not noted | | | Died 20th day; encephalitis on postmortem examination |
| 12 | Baker & Noran [34] | 2 mo. | M | Coma; convulsion | 30th | ? | 189 (all mononuclear) | 126 | Died |
| 13 | Baker & Noran [34] | 45 | F | Meningitis | 3rd | Inc. | ? | ? | Died 6th day |
| 14 | Baker & Noran [34] | 50 | M | Delirium | 3rd | ? | ? | ? | Died 4th day; encephalitis at autopsy |
| 15 | Baker & Noran [34] | 29 | M | Stupor; right hemiplegia | 9th | ? | N | 59 | Recovered; residuals of hemiplegia and encephalitis |
| 16 | Glendy & Hankins [28] | 26 | M | Mental confusion; pathological reflexes | 3rd | ? | 33 | Inc. | Recovered |
| 17 | Glendy & Hankins [28] | 22 | M | Stiff neck; L. ext. strabismus | 3rd | 160 | N | N | Recovered |
| 18 | Turner [29] | ? | | Transverse myelitis | ? | Not reported | | | Recovered |
| 19 | London & Laven [21] | 24 | M | Transverse myelitis | 22nd | ? | 183 (95% P) | 110 | Recovered in 1 wk. |
| 20 | Norris and others [30] | ? | | Myelitis | ? | ? | 182 (L) | 40 | Recovered |
| 21 | Holmes [31] | 65 | M | Encephalomyelitis | 7th | 100 | 19 (L) | 120 | Recovered in 8 days |
| 22 | Holmes [31] | 53 | M | Encephalomyelitis | 7th | 130 | 0 | 80 | Recovered in 9 days |
| 23 | Holmes [31] | 26 | M | "Lymphocytic" meningitis | 3rd | 130 | 64 (L) | 90 | Recovered in 8 days |
| 24 | Holmes [31] | 13 | M | "Lymphocytic" meningitis | 3rd | 300 | 100 (50% L) | 30 | Recovered in 4 wk. |
| 25 | Holmes [31] | 37 | M | "Serous" meningitis | ? | 300 | N | 80 | Recovered in 3 days |
| 26 | Holmes [31] | 29 | F | Encephalitis | 3rd | 160 | 0 | 30 | Recovered in 2 wk. |
| 27 | Suchett-Kaye [4] | 40 | M | Transverse myelitis | 14th | 200 | 220 | 320 | Died 24 days after onset of myelitis |
| 28 | Fowler [20] | 37 | F | Left hemiplegia | 5th | 120 | 220 (95% P) | 125 | Died in 2 days |
| 29 | Chang [16] | 28 | F | Myelitis; Guillan-Barré-like syndrome | 18th | ? | 1 | 154 | Recovered after 2 mo. |
| 30 | Fiaschi & Sarteschi [15] | 47 | ? | Encephalitis right hemiplegia | 13th | Not done | | | Died in 3 days |
| 31 | Fiaschi & Sarteschi [15] | 18 | ? | Transverse myelitis | ? | N | 26 | Inc. | ? |
| 32 | Sticca & Toscano [32] | 4 | F | Meningitis | ? | ? | Inc. | Inc. | Subsided rapidly |
| 33 | Sticca & Toscano [32] | 8 | M | Meningitis | ? | ? | Inc. | Inc. | Subsided rapidly |
| 34 | Scarlini [2] | 53 | M | Meningitis | 15th | ? | 9 | 2+ | Recovered on 20th day; residual of clouded sensorium, headaches |
| 35 | Lazzari † | 17 | M | Stupor; paralysis | 23rd | Not done | | | Recovered |
| 36 | Gsell & Engel [33] | 30 | M | Serous meningitis | ? | 185 | 17 | 0 | Recovered |
| 37 | Ingleby [5] | 58 | F | Encephalitis | 3rd | Not done | | | Died 3rd day of illness; encephalitis at autopsy |
| 38 | Yesnick | 34 | F | Meningo-encephalitis | 14th | 230 | 81 (85% L) | 44 | Recovered in 4 days |

* P, polymorphonuclear cells; L, leucocytes; N, normal.

† Cited by Scarlini.[2]

endocarditis, which may have profound central nervous system effects.[6] The case reports of Keizer[7] were omitted because three of the children had negative chest plates; the fourth had bloody spinal fluid, something unique among all the cases reviewed. The case report of Hassin and Broder[8] (included in Scarlini's[2] series of cases) is omitted because no mention of pneumonia is made anywhere in their protocol. The case reports of Schacter[9] are omitted because of the long intervals between the pneumonic episodes and the presumptive associated central nervous system complications: The two occurred years apart. The case of Kneeland and Smetana[10] (also cited by Scarlini[2]) is omitted because of the bizarre course of the patient's illness and the negative spinal fluid findings; the authors admit that no diagnosis was actually established.

It is perhaps of some interest that no cases have been reported in the American and British literature since 1951, while a number of recent reports have appeared in the European literature, predominantly from pediatric clinics. Unfortunately, as noted above, some of these reports are not adequately documented.

## COMMENT

*Incidence.*—In the present review of the literature only 19 case reports of central nervous system complications of primary atypical pneumonia were found in the American literature. Most of these reports appeared during the Second World War, when the disease was seen in epidemic proportions at various military installations. No case reports have appeared since 1951. This apparent low incidence compares favorably with the incidence of encephalitis complicating pneumonia in general about 25 years ago. Adler[11] found 11 such cases at the Boston City Hospital in 1928-1938. She estimated that about 18,000 cases of pneumonia had been treated there during that time. The incidence of encephalitis complicating pneumonia in her series would be about 0.06%. One may speculate that the actual incidence of encephalitis is perhaps higher than these figures indicate. Thus von Ravensway and co-workers[12] listed headache in 48% and "meningeal signs" in 0.4% of their reported cases of primary atypical pneumonia. Spinal punctures were not done. On the other hand, a patient with atypical pneumonia may show disorientation and clouded sensorium and have normal spinal fluid findings.[27]

*Age.*—The age of the patients varied from 1 month to 65 years.

*Sex.*—About two-thirds of the reported cases occurred in males. This predilection for males may be more apparent than real, because many of the case reports came from military installations, with almost exclusively male populations.

*Onset of the Complication with Relation to Onset of the Pneumonia.*—The onset varied from the 3rd day of the illness to the 30th day after the onset of the illness. In four cases the patients had been afebrile from two days to two weeks preceding the onset of the complication. The time of onset appeared to have no prognostic significance. It is difficult to evaluate Schacter's[9] reported cases in which neurologic disease occurred in children with a history of bronchopneumonia years previously. A causal relationship between the neurologic disease and the pneumonia is assumed. The validity of this assumption is not substantiated.

*Types of Lesions Observed.*—These are summarized in Table 2. The classification is based on the predominant manifestation of the complication.

It is of interest that nearly all of these complications were nonsuppurative. The outstanding exception is the case of Campbell and co-workers,[13] who found small abscesses in the dorsal spinal cord. No case of brain abscess appears to have been reported complicating viral pneumonia. There is one case report of bacterial meningitis complicating primary atypical pneumonia due to the Meningococcus.[12]

*Spinal Fluid.*—The fluid was always clear. Pressure varied from normal up to 300 mm. Total protein varied from normal to 326 mg. per 100 cc. Cell counts varied from 0 to

350; the differential counts varied from all lymphocytes to 95% polymorphonuclear cells. Unfortunately, spinal fluid examinations were not done in some cases and not reported in others. From the available data there appears to be no fixed pattern among the spinal fluid findings: the pressure may be increased with no change in protein or cells; pressure can be normal with increased protein and cells; there may be an increase in cells only, and these cells may be predominantly lymphocytes or polymorphonuclear, and, finally, there may be an increase in protein without increase in cells, producing spinal fluid like that in Guillan-Barré disease. Cultures have been invariably sterile.

*Prognosis.*—There were 12 deaths among the 38 reported cases, a gross mortality of 32%. At least three of the reported deaths occurred in patients whose illnesses may well have been other than primary atypical pneumonia. Thus, the cases of Ingleby [5] and Suchett-Kaye [4] have already been cited. One of the cases of Fiaschi and Sarteschi [15] (Case 30 in Table 1) may be similarly questioned. If one eliminates these questionable cases, one obtains a mortality rate closer to 25%. Death from central nervous complication did not appear to be influenced by age, time of onset with respect to day of illness of the pneumonia, or type of complication. The number of cases, however, is too small to justify anything more than an impression. If a patient survives the complication, the chance for complete recovery is excellent. Of the 30 patients who recovered only 3 had residuals: 1 when last seen, 3 months after his illness, had ataxia and mental deterioration (Case 10); 1 had hemiparesis and mental changes (Case 15), and 1 had a clouded sensorium and headaches (Case 36). The length of follow-up in the latter two cases is not stated.

TABLE 2.—*Types of Central Nervous System Lesions with Pneumonia*

| Lesion | Cases, No. | Deaths |
|---|---|---|
| Meningoencephalitis | 24 | 8 |
| Transverse myelitis | 5 | 1 |
| Hemiplegia | 4 | 2 |
| Ascending paralysis | 4 | 1 |
| Cranial nerve palsy* | 1 | 0 |

* Rosen [14] mentions two cases of ocular muscle paralysis complicating viral pneumonia discussed at a Walter Reed General Hospital Conference in 1943. These are not listed in Table 1 or Table 2 because of lack of appropriate data.

*Treatment.*—A Drinker respirator appeared to be life saving in a patient with ascending spinal cord paralysis.[16] In the absence of respiratory paralysis only general nursing care is indicated. Antibiotics and chemotherapeutic agents have not been demonstrated to have a beneficial effect.

*Nature of the Complication.*—The etiology of the encephalitis complicating primary atypical pneumonia has been the subject of much speculation and relatively little laboratory and clinical investigation. Noran and co-workers,[17] after studying this problem both clinically and experimentally, concluded that encephalitic complications of pneumonia are the result of intravascular clotting within the central nervous system. This clotting is presumed to be due to substances liberated by the lung during the pneumonitis. This hypothesis has neither been confirmed or denied since its publication. Ingleby's [5] patient, upon autopsy study, showed inclusion bodies in the brain and in the bronchial mucosa. As noted above, this patient had bacterial endocarditis. However, even if one assumes that the patient actually did have primary atypical pneumonia and complicating encephalitis, these inclusion bodies have no specificity, since they also occur in bacterial disease (e. g., pertussis) and tumors (meningiomata).* Attempts at isolating a specific virus by inoculating brain and lung tissue obtained at autopsy failed.† Serologic studies for evidence of a specific viral encephalitis were negative in two cases.‡ From the available facts very little can be said about the cause of the central nervous system changes. Indeed, this uncertainty about the nature of the encephalitis complicating pneumonia is applicable to encephalitis complicating fever, exanthem, and infections in general.§

* Reference 18, p. 65.
† References 19 and 20.
‡ Reference 21 and the present report.
§ Reference 18, p. 96.

These encephalitides appear to constitute a histopathologic group with no etiologic or histologic specificity.[22]

SUMMARY

A case of meningoencephalitis complicating primary atypical pneumonia is reported, and the literature is reviewed.

Central nervous system complication of primary atypical pneumonia is rare, probably occurring in less than 0.1% of the cases.

This complication is nearly always non-suppurative.

The complication oftenest manifests itself in the form of meningoencephalitis, but it may involve the meninges, brain substance proper, spinal cord, or cranial nerves, individually or collectively.

The complication has not been reported before the third day after onset of the pneumonia; it may occur as late as two weeks after defervescence and apparent clinical recovery from the pneumonia.

The over-all mortality rate in the collected cases was 32%.

If the patient survived the complication, recovery was generally rapid and complete; only three patients were reported to have residuals, one as long as three months after the illness.

The exact nature of the noxious agent (viral, toxic, allergic, vascular) remains unknown, nor does it appear to have any histologic specificity.

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# Treatment in Internal Medicine

## TREATMENT OF TUBERCULOSIS

KARL H. PFUETZE, M.D.
and
EUGENE J. DesAUTELS, M.D., Chicago

DURING the past decade since the advent of streptomycin, changes in the treatment of tuberculosis in its various forms have occurred with almost bewildering rapidity. This period of transition has been marked by considerable difference of opinion as to the antimicrobial regimen of choice, duration of treatment, indications for surgical intervention, etc.* However, there is general agreement that specific antimicrobial therapy is indicated in all forms of active tuberculosis, regardless of the organs or system involved. The treatment of pulmonary tuberculosis will be considered first.

### PULMONARY TUBERCULOSIS

#### Drug Therapy

The drugs most commonly used today are isoniazid, streptomycin (or dihydrostreptomycin), and sodium aminosalicylate (PAS-sodium). Viomycin is used much less often. Pyrazinamide in combination with isoniazid is another drug combination which may emerge after further investigation as a significant addition to the armamentarium.[10]

*Streptomycin Sulfate.*—This is one of the most effective antimicrobial agents we have for tuberculosis. It should not be used alone because of the emergence, after a few months, of streptomycin-resistant tubercle bacilli. The chief toxic manifestations are vestibular damage and allergic reactions. Formerly, when dosages of 1 to 2 gm. daily were employed for prolonged periods, vestibular disturbance occurred very frequently. However, the presently recommended dosage of 1 gm. twice weekly practically eliminates this hazard. When allergic rash occurs, it is sometimes possible to desensitize the patient by gradually increased daily doses, starting with 0.05 to 0.1 gm. streptomycin. If this fails, dihydrostreptomycin may often successfully be substituted.

*Dihydrostreptomycin.* — Dihydrostreptomycin sulfate, a hydrogenated derivative of streptomycin, has the same therapeutic efficacy as streptomycin, and the recommended dosage is the same. It may be used as a substitute for patients who are hypersensitive to streptomycin. Unlike streptomycin, it causes little or no vestibular damage, but it has the distinct disadvantage, when given daily in doses of 1 to 2 gm., of causing deafness in some patients. This deafness may be partial or total and is irreversible. In the recommended dosage of 1 gm. twice weekly, appreciable deafness rarely occurs. However, physicians who wish to use dihydrostreptomycin sulfate may employ it in 0.5 gm. doses, combined with 0.5 gm. of regular streptomycin given twice weekly. Such combined preparations are readily available and practically eliminate the danger of either vestibular damage or deafness.

*Isoniazid.*—Isoniazid was introduced in 1952 and has proved to be perhaps the most potent antituberculosis agent. It is inexpensive and can be given orally and, when necessary, parenterally. It is strikingly effective in reducing cough, expectoration, fever, and malaise and in improving the weight and general well-being of the patient. In the recommended dosage of 3 to 5 mg. per kilogram body weight, toxic side-reactions occur but seldom. These consist chiefly of peripheral

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Medical Director, Chicago State Tuberculosis Sanitarium, and Clinical Professor of Medicine, University of Illinois (Dr. Pfeutze), and Chief, Tuberculosis Service, V. A. Hospital, Hines, Ill. (Dr. DesAutels).

* References 1-9.

neuritis, hyperreflexia, and positional hypotension. The latter two are not serious and usually disappear or subside without the drug being discontinued. When peripheral neuritis occurs, the dosage should be lowered or the drug discontinued until symptoms subside. Pyridoxine hydrochloride (vitamin $B_6$) in doses of 50 to 100 mg. t. i. d. has been found to be helpful in the management of many such cases. Some investigators advocate higher doses of isoniazid, though, in our opinion, there is little evidence to date that the larger doses need be employed, except in miliary and meningeal tuberculosis. Isoniazid has been reported to cause convulsions, especially in patients with a history of previous convulsive disorders. In such patients it should be used with great caution and barbiturates should be added to the regimen. Psychotic episodes have also been reported following its use, but fortunately such incidents are relatively rare and occur chiefly in patients with a previous history of emotional instability and disturbance.

Like streptomycin, isoniazid when used alone results in the emergence of isoniazid-resistant organisms usually after two to four months. For this reason, it is seldom recommended as single drug therapy. However, as Muschenheim has pointed out, the good results in miliary tuberculosis treated with isoniazid alone,[11] and evidence accumulated thus far in investigations with pulmonary tuberculosis, indicate that correlations between in vitro drug resistance and in vivo response to treatment are less clearly defined than with streptomycin.[8] Nevertheless, it is not infrequently observed that progression of disease occurs in patients whose organisms are resistant to isoniazid. Until further investigation more clearly defines the type of case in which isoniazid can and perhaps should be used alone, we feel that it should be combined with streptomycin, or PAS, or both.

*Aminosalicylic Acid.*—PAS remains an important antimicrobial agent in tuberculosis. Though not as potent as isoniazid or streptomycin, when used in combination with either or both of these drugs it delays the emergence of organisms resistant to them, and thus it substantially prolongs their therapeutic effectiveness. It is usually given in the form of PAS-sodium. It is also available as calcium-PAS and potassium-PAS. The dosage most commonly accepted as optimal is 12 gm. daily (or 16 gm. of sodium-PAS) divided into three or four doses. When a low-sodium intake is desired, the calcium-PAS or potassium-PAS may be used.

The chief complaints encountered from the use of PAS are allergic manifestations and gastrointestinal disturbances such as diarrhea, nausea, and abdominal distress. Unless the intestinal symptoms become severe or interfere with adequate nutrition, the patient is encouraged to continue with the drug. Allergic manifestations may occasionally be severe, with exfoliative dermatitis. Lichtenstein and co-workers[12] have reported toxic side-reactions simulating infectious mononucleosis or hepatitis. In such instances the drug should be discontinued immediately. Antihistaminics are often helpful, and sometimes desensitization may be attempted later. On occasion, steroid hormone therapy has been successfully employed. In critically ill patients a lyophilized form of PAS can be given parenterally to good advantage for relatively short periods of time. It can also be given this way as protection for surgical intervention in selected cases.

*Viomycin.*—Viomycin sulfate has a useful but limited place in the treatment of tuberculosis. It is most often used in patients whose organisms are resistant to streptomycin and isoniazid or in patients who cannot tolerate these drugs.[13] The usual dosage is 2 gm. twice weekly. It is preferrable to use it combined with another drug to which the organisms are still sensitive. Some surgeons employ it two or three weeks before and several weeks after resective pulmonary surgery as an extra protective measure.

*Pyrazinamide.*—Pyrazinamide (PZA) is now available for use in hospitals only. Used alone it is effective for no longer than six or eight weeks. Used in combination with isoniazid it may be very effective therapy for

many months.[10] However, the hazards of hepatic toxicity are real and preclude a recommendation for its general use at this time. Liver-function studies must be performed before and at frequent intervals during administration of this drug. It must be discontinued immediately upon evidence of hepatic toxicity. The usual dosage of pyrazinamide is 30 to 35 mg. per kilogram of body weight daily, divided into three doses, the total dose not to exceed 3 gm. daily.

*Recommended Regimens.*—At the present time there is no generally accepted "optimum" antimicrobial regimen. However, there is agreement on the following general principles: (1) Chemotherapy should consist of at least two drugs given concurrently; (2) they should be given continuously, without interruption, and (3) they should be given for at least a year even in minimal cases, and much longer in the more advanced cases.

In our opinion any one of the following regimens constitutes good antimicrobial therapy for the various forms of tuberculosis, except miliary and meningeal tuberculosis.

1. Isoniazid, 3 to 5 mg. per kilogram of body weight daily in three divided doses, plus PAS-sodium, 16 gm. daily in three or four divided doses.
2. Isoniazid as above plus streptomycin sulfate 1.0 gm. i. m. two to three times weekly.
3. Isoniazid plus streptomycin sulfate plus PAS-sodium, each in doses as given above.

In these dosages, serious side-reactions seldom occur, even when continued for two to three years, as is often necessary in far-advanced cases. In acutely ill patients streptomycin may be given daily for the first three or four weeks. Recent studies in the United States and England indicate that the combination of streptomycin and PAS which has enjoyed such wide popularity in the last few years is not as effective as the three above regimens, particularly in advanced pulmonary tuberculosis with large cavities.

### Other Therapy

Rest is still important in the treatment of tuberculosis. Prior to the advent of chemotherapy strict bed rest was the order of the day. In the past few years, however, strict bed rest has usually been limited only to the acutely ill patients. At present, limited activities may be permitted when the fever and other signs of toxicity have subsided. There is still difference of opinion as to what constitutes an "adequate" rest regimen in the management of pulmonary tuberculosis, but unquestionably with antimicrobial therapy the patient's activities may be increased safely much more rapidly than in the past. Diet should be varied, properly balanced, well prepared, and appetizingly served. The caloric intake should be such as to enable the patient to regain and maintain his ideal weight. Supplementary vitamins may be indicated in selected cases, especially during the first few weeks of illness.

Collapse therapy enjoyed a tremendous vogue until a few years ago, when combined chemotherapy and resective surgery came into general use. Since then its popularity has declined steadily. Collapse therapy includes several procedures, such as (1) artificial pneumothorax, (2) pneumoperitoneum, (3) phrenemphraxis (crushing of the phrenic nerve), and (4) major surgical collapse procedures, such as thoracoplasty. Artificial pneumothorax is now used very little if at all by most physicians treating pulmonary tuberculosis. It has certain dangers and complications, such as (1) perforation of the lung, (2) air embolism, (3) pleural effusion resulting in thickened pleura and marked reduction of lung function, (4) tuberculous empyema, (5) bronchopleural fistula. It is seldom indicated, and its success in a given patient is dependent upon the judgment with which the case is selected and the skill with which it is subsequently managed. Pneumoperitoneum is used much less now than formerly, but its popularity varies greatly in the different geographic regions in the United States. It may be helpful in controlling lesions in the lower and midlung fields, but it is of little value in closing chronic apical cavities. Its main value is probably as an adjunctive procedure in far-advanced bilateral cases to prepare the patient for surgical intervention later. Phrenemphraxis, the crushing of the phrenic nerve,

with resulting paralysis of the hemidiaphragm was popular a decade or more ago. In the past few years there has been a sharp decline in its use. At present it is seldom if ever indicated. Thoracoplasty, though largely replaced by resectional surgery, still retains a significant place in the treatment of tuberculosis. Certain variations and modifications of this procedure have been introduced from time to time. These include the use of an extraperiosteal wax pack or other prosthesis and are designed to produce an adequate collapse while conserving the maximum of respiratory reserve. In selected cases where resectional surgery is not feasible or carries too much risk, thoracoplasty may be the treatment of choice.

Surgical resection in pulmonary tuberculosis has come into common use in recent years, owing chiefly to antimicrobial therapy, advances in anesthesia, and improvements in surgical techniques. Depending on the amount, character, and location of the residual disease, pneumonectomy, lobectomy, segmental and the so-called "wedge" resection, or combinations of these procedures may be employed. At present there is considerable controversy as to the exact indications for its use.[14] However, there seems to be general agreement on the following indications for resection: (1) the so called "destroyed" lung, for example, a case in which a lobe or lung is almost completely destroyed by the disease process; (2) severe bronchial stenosis; (3) cavity persisting after thoracoplasty (thoracoplasty failure); (4) residual cavities persisting after six months or more of chemotherapy; (5) tuberculoma, and (6) bronchiectasis associated with tuberculosis. The removal of tiny nodular foci by wedge resection enjoyed a certain vogue a few years ago but is seldom employed now. Whether the larger residual nodular foci should be surgically removed or whether they may be successfully treated with long-term combined chemotherapy alone is controversial at this time. Further long-term studies will be required before this question can be resolved.

Surgical intervention, if indicated, should be performed at the optimal time during the management of the case. For this reason, the clinician should have consultation at intervals with a competent chest surgeon who is experienced in tuberculosis. In general, surgery should be withheld until maximum resolution of reversible disease has occurred and residual disease is stable and consists of irreversible necrotic or destroyed tissue, e. g., cavities. This status often occurs by the sixth to eighth month in patients receiving antimicrobial therapy for the first time. In this way, the extent of surgery with its concomitant loss of tissue may be held to the minimum consistent with a reasonably good long-range prognosis. Early in the course of treatment adequate roentgenologic studies should be done to demonstrate the maximum involvement of disease. For this purpose roentgenograms should include posteroanterior, lateral, and, at times, apical views and tomography. These studies should be repeated again before surgery to demonstrate the extent and location of the residual disease. In this way, the clinician and chest surgeon can best determine the type and extent of surgery indicated and whether it is feasible. In some cases pulmonary-function studies are also essential in reaching a decision. Antimicrobial therapy should be continued for many months after surgery and after discharge from the hospital. The optimum duration of antimicrobial therapy is actually undetermined as yet, but we recommend that it be continued for at least six months in the most favorable cases and up to two years or more in others, depending upon the amount of residual disease present after surgery.

### OTHER FORMS OF TUBERCULOSIS

Acute miliary tuberculosis responds amazingly well to antimicrobial therapy. Survival rates of 90% to 100% are often reported. One group has even reported 100% survival rate among 12 consecutive patients treated with isoniazid alone.[11] But, remarkable as this series is, no one at present recommends isoniazid alone as the regimen of choice in the treatment of miliary tuberculosis. The regimen employed by us consists of isoniazid daily, 6 to 10 mg. per kilogram body weight

in three divided doses for the first two or three months, after which the dose is reduced to 5 mg. per kilogram per day; streptomycin 1 gm. daily for the first three months, then twice weekly, and 16 to 20 gm. of PAS-sodium per day (12 to 15 gm. of aminosalicylic acid) in four divided doses. Chemotherapy should be continued for at least 18 months. During the period that isoniazid is given in the higher dosages, pyridoxine hydrochloride in doses of 100 mg. t. i. d. is recommended to prevent peripheral neuritis.

Tuberculous meningitis likewise responds very well to chemotherapy. Survival rates of 80% to 90% are not uncommon.[15] The antimicrobial regimen of choice is essentially the same as that for miliary tuberculosis and should be continued for 18 months to 2 years. We do not recommend the routine use of intrathecal therapy, for we believe it is seldom indicated and may be harmful. For best results it is imperative that chemotherapy be instituted immediately after a presumptive diagnosis is made based on history, physical signs and symptoms, and spinal fluid findings. If the patient's condition does not permit oral medication, the isoniazid and PAS should be given parenterally until he becomes rational.

In genitourinary tuberculosis the antimicrobial regimen of choice at present is isoniazid, streptomycin, and PAS in dosages as recommended for pulmonary tuberculosis. The chemotherapy should be continued for at least a year and in cases of advanced destructive renal lesions for much longer. Tuberculosis of the prostate, epididymis, and bladder also responds well to this regimen. In patients with appreciable kidney impairment the dosage of all antimicrobial agents should be adjusted to prevent excessive blood levels with possible attendant toxic side-effects. Therapy should be started in a hospital and in the severer cases should be continued there if possible. Lattimer [16] reports that antimicrobial therapy is often definitive in such cases. The urologist can best decide when and if surgical intervention is required and should be consulted at frequent intervals in these cases. In pelvic tuberculosis in the female combined chemotherapy for a year or more is often definitive. Surgical intervention is not indicated in such cases except to remove residual tuberculous abscesses.

Tuberculosis of the bones and joints: We recommend the same antimicrobial regimens for this as for pulmonary tuberculosis. The chemotherapy should be continued for at least a year and often for much longer, until adequate healing has occurred. Early tuberculosis of the joints often responds well, and chemotherapy may be definitive without operation except for biopsy to establish the diagnosis. Surgical drainage, excision, and debridement of grossly necrotic tissue with or without fusion will often be necessary before secure healing is attained. The type and extent of surgery can best be determined by the orthopedist, and the optimum time for surgical intervention should be decided in collaboration with the phthisiologist.

Pleurisy with effusion: In the vast majority of cases tuberculosis is the cause of so-called "idiopathic" pleurisy with effusion in a patient whose Mantoux test is positive and in whom no other etiology can be found. Treatment consists of aspiration of the fluid, bed rest for a few months, and combined antimicrobial therapy for at least a year, as is recommended for active pulmonary tuberculosis. The importance of adequate treatment for this condition can not be overemphasized, since in 25% to 65% [17] of these cases, if they are not treated with antimicrobial therapy, active tuberculosis develops in some part of the body within five years.

Tuberculous pericarditis, in the early acute phase, often responds well to prolonged antimicrobial therapy, though aspiration (relatively early) may be required to relieve tamponade. In other cases, however, pericardectomy may be necessary to relieve severe constrictive pericarditis which sometimes develops during the later stages of the healing process.

Tuberculosis of peripheral lymph nodes, particularly early acute adenitis, usually responds well to antimicrobial therapy. We have had rather consistently good results in this type with isoniazid alone. Chronic

caseous nodes, sinuses, and fistulae may require excision of all necrotic tissue in addition to prolonged combined chemotherapy.

Other forms of tuberculosis involving such organs and tissues as the middle ear, mouth, larynx, gastrointestinal tract, peritoneum, and skin usually respond to and should be treated by antimicrobial therapy for a year, with use of any one of the three regimens recommended for pulmonary tuberculosis. For tuberculosis of the adrenal glands isoniazid, streptomycin, and PAS are recommended, plus replacement hormonal therapy as indicated.

Tuberculosis in childhood: During the past two years there has been a growing conviction that all children with active tuberculosis should receive antimicrobial therapy. We are in agreement with this principle. Complications such as miliary and meningeal tuberculosis are practically eliminated in such cases when isoniazid is employed. The regimen should include 5 mg. isoniazid per kilogram of body weight daily combined with 0.5 to 1.0 gm. (depending upon body weight) of streptomycin sulfate twice weekly, or 0.25 to 0.5 gm. of PAS-sodium per kilogram of body weight daily, or both, and it should be continued for at least one year. In infants under 2 years of age who have a positive Mantoux test, we recommend isoniazid alone, given daily 5 mg. per kilogram body weight in three divided doses, for one year.

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## Case Reports

# AMEBIC ABSCESS OF THE LIVER PRESENTING IN THE SUBSCAPULAR AREA

JAMES J. DIAMOND, M.D.
and
ROBERT A. SCRIBNER, M.D., Reading, Pa.

THE PURPOSE of this report is to present an unusual case of hepatic abscess due to infestation by Endamoeba histolytica. It is unusual in that the abscess spontaneously ruptured and dissected its way extrapleurally up into the region of the inferior angle of the right scapula, where it presented itself as a soft-tissue mass in the subcutaneous level. When a search of the literature failed to reveal any similar cases, even through the past decades when amebic abscess was a far commoner disease, it was elected to present the essential details of this case history to the medical profession.

### REPORT OF CASE

A 24-year-old white man was admitted to the hospital because of a mass in the right subscapular region, which had appeared over a three-week period.

Three months prior to admission he had fallen against a truck handle, striking his lower right rib cage. Minimal symptoms of slight pain and tenderness in the area had persisted annoyingly until admission.

Three weeks prior to admission he noticed the appearance of a small swelling in the right subscapular area. At the same time, he noticed the onset and gradual increment of fatigue, anorexia, weight loss, night sweats, and tenderness in the region of the mass.

Careful interrogation could elicit no history compatible with amebiasis in any of its many forms. No positive evidence of any gastrointestinal disorder at any time in his past life could be obtained. No pleuropulmonic symptoms were present.

Examination revealed a thin, pallid man in no acute distress but appearing chronically ill. Physical findings, briefly, consisted of the mass and signs of

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From the Surgical Service, St. Joseph's Hospital.

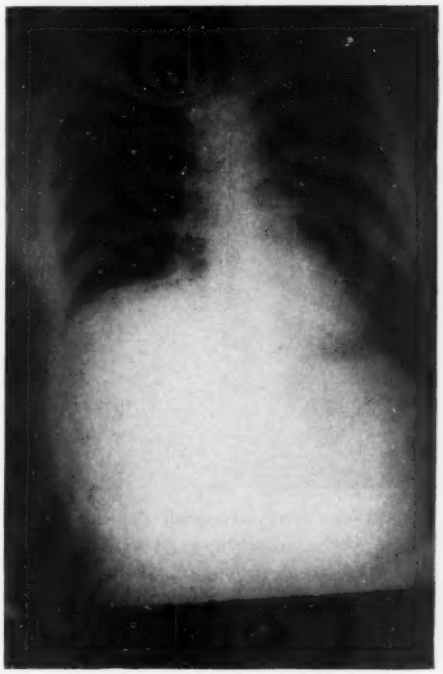

Fig. 1.—Chest x-ray on admission, showing elevated diaphragm. A vague soft-tissue mass is seen over posterolateral rib cage, below scapula.

an elevated right diaphragm. The mass measured 6.0 by 10.0 by 4.0 cm. and lay on the posterolateral aspect of the right chest wall. It was warm, slightly tender, and firm. Its upper pole overlay the inferior angle of the scapula, and from that point the mass extended downward and outward to occupy the structures normally placed in the posterior axillary fold. On percussion, hepatic dullness extended to the sixth rib anteriorly and showed no excursion. The right lungbase was dull to percussion and devoid of breath sounds. None of several clinicians could discern the liver edge, even though the patient was quite thin.

His temperature was 100 F; pulse rate, 76; respirations, 26. A moderate anemia and leucocytosis were present. Urinalysis was normal. A chest roentgen study showed the diaphragm elevated to the anterior shadow of the fourth rib. The lung fields were clear. Lateral study could show no link

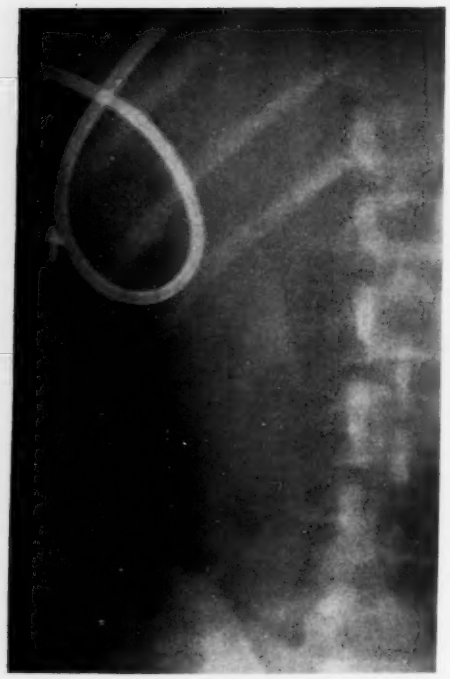

Fig. 2.—Intravenous urogram, showing flattened pelvic shadow.

between the elevated diaphragm and the mass on the chest wall (Fig. 1).

*Course.*—While further studies of the subdiaphragmatic disorder were carried out, the mass was biopsied, to determine whether it was a tumor. The lower edge of the mass, which lay deep to the latissimus dorsi muscle, was exposed, and it was seen that it was a faintly fluctuant abscess; incisional drainage was effected, with removal of 50.0 cc. of sanguinopurulent matter. A rubber dam was inserted, and the patient was returned to the ward.

Chlortetracycline (Aureomycin), 1.5 gm. daily, had no effect on the fever, which rose to 103 F daily. The wound drained profusely, but repeated studies for fungi, bacteria, amebae and acid-fast organisms were negative (smear, culture, animal inoculation). Supportive care was intense but noncurative.

An intravenous urogram revealed a flattened pelvic shadow, which suggested extrinsic pressure (Fig. 2).

A barium meal revealed an inconstant pyloric niche which was considered as possible evidence of an ulcer; the subdiaphragmatic disorder then was considered the result of a perforated ulcer, despite the unlikely past history.

A 12 F. catheter was threaded into the draining sinus, and under fluoroscopic visualization it was seen to follow a course upward to the inferior angle of the scapula, then turn sharply downward, deep to the rib cage, and proceed for 8 in. more before encountering resistance. Approximately 300 cc. of anchovy-sauce-appearing pus was aspirated. Then 240 cc. of sodium iodomethamate U. S. P. (Neo-Iopax) was injected into the catheter, and roentgenograms were taken from several angles. These revealed a pocket measuring 6.0 by 6.0 by 8.0 cm. under the right diaphragm and a connecting sinus which ran up the inner surface of the ribs to the subscapular area, where it entered the submuscular abscess (Fig. 3).

The aspirated material was centrifuged at 3300 rpm for 15 minutes, and the buffy supernatant fluid revealed many active trophozoites of E. histolytica.

Under standard doses of chloroquine (Aralen), carbarsone, and oxytetracycline (Terramycin) the

Fig. 3.—Cavity demonstrated by sodium iodomethamate instillation. Left lateral decubitus position; film overexposed to show fluid level.

Fig. 4.—Abscess cavity as visualized 96 hours after film shown in Figure 3. Left lateral decubitus position.

patient showed no response. Adequate parenteral fluids and multiple transfusions were given, with little visible effect. Two catheters, one 12 F. and one 18 F., were threaded into the abscess cavity, and continuous irrigation with oxytetracycline (1.0 gm. per liter) was instituted. The larger catheter drained the grossly bloody debris and pus from the cavity. By 36 hours, little debris was recognizable in the return fluid and the instilled volume was identical with the volume of drainage. The temperature dropped to normal by 36 hours, never rising above 98.4 F thereafter. Marked clinical improvement was evident. At 96 hours, repeat visualization of the abscess cavity with sodium iodomethamate revealed a diminishment of volume to approximately 50 cc.; at 10 days, the volume was 15 cc., and thereafter only the closing sinus could be visualized. The sinus healed completely by the 17th day. When seen one month after discharge, the patient had gained 35 lb. and was entirely symptom-free.

## COMMENT

Since the features of hepatic amebiasis are well known, only four facets of this case appear significant enough to warrant comment.

A. The role of trauma in the production of amebic abscess of the liver has not received wide credence or consideration. Berne [1] reported three cases in which the acute development of amebic liver abscess followed trauma to the liver area, in persons not previously manifesting any symptoms identifiable with amebic infestation. This possibility has also been mentioned by Faust,[2] who would postulate an amebistatic factor in the liver which is able to destroy the organisms in the portal system until some added insult such as trauma lowers the resistance of some portion of the hepatic substance and provides a medium for proliferation of the amebae. Whether or not the chest injury incurred by our patient actually caused local hepatic necrosis is only conjecturable. The site of trauma was placed over the liver by him, and the course of events has given us pause, in view of the experiences of the cited authors.

B. Craig [3] summarized 142 instances of rupture encountered in 562 cases of amebic abscess of the liver and found that about 75% of ruptures occur into the right lung or pleural cavity, pericardium, or peritoneal cavity. Other rupture sites include the following: subhepatic and subphrenic spaces, lumbar region, stomach, duodenum, colon, vena cava, kidney, and bile ducts. He does not describe any route that coincides with the one demonstrated in our case. Clark [4] saw one case with transdiaphragmatic rupture out into the body wall, which parallels this case more closely than any other reported case.

C. The catheter instillation of oxytetracycline into the abscess as a therapeutic technique has indelibly impressed all who observed this case as the difference between success and failure in this one case. The use of this irrigation coincided with a remarkable amelioration in the clinical picture of a patient in critical condition. This is not to say that therapeutic effect was attributable to the antibiotic contained in the solution. Sherry and his associates [5] were able to produce a similar effect with instillations of streptokinase-streptodornase solution, and it

seems logical to expect a decreased absorption of toxic, necrotic matter into the systemic circulation from the abscess when irrigation with any suitable solution has removed large amounts of debris from the cavity. Dr. Alton Ochsner was consulted on this point, and he attributes greater therapeutic effect to the irrigations than to the antibiotic contained in the solution.*

D. The flattening of the renal pelvis shadow on urographic study served to aid and to confuse the diagnosis. The history of trauma caused us to consider a renal injury with massive hematoma formation as the cause of the elevated diaphragm. The possibility of perirenal abscess also entered the picture at one time, particularly when repeated negative cultures in the first few days introduced tuberculosis as the possible cause of an abscess. Coppridge and his associates [6] reported one case, and cited two others of Yates, in which a liver abscess caused roentgenographic defects in the kidney. This unusual finding was a distracting feature until the ultimate diagnosis was established.

SUMMARY

A case of amebic abscess of the liver is presented. Its unusual features are a rare site of presentation, on the right subscapular area of the chest wall, an uncommon urographic deformity, due to extrinsic pressure by the abscess mass, and a dramatic response to irrigation therapy. The role of trauma in the genesis of liver abscess is briefly considered, and an irrigation technique is described, without the suggestion that it be considered an advance in the therapy of amebic abscess.

* Ochsner, A.: Personal communication to the authors.

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# News and Comment

## ANNOUNCEMENTS

**Fellowships in Cardiology and Hematology.**—Two fellowships are available at the Fort Miley Veterans Administration Hospital, 42nd Ave. and Clement St., San Francisco. These fellowships are by appointment from Stanford University School of Medicine, and each bears a stipend of $300.00 per month. The appointments are for one year and will be renewable for an additional year. The applicants must be graduates of a Class A medical school, with preference given to candidates who have adequate previous training in clinical medicine.

One is a fellowship in cardiology under the auspices of the San Mateo County Heart Association, beginning July 1, 1956, and will provide an opportunity for clinical investigation or basic research in the field of cardiovascular disease. The other fellowship is in hematology, beginning on the same date, under the auspices of the National Institutes of Health, and will provide opportunity for clinical investigation or basic research in the field of hematology and tumor chemotherapy.

**A. M. A. Annual Meeting, Chicago, June 11, 1956.**—The 105th Annual Meeting of the American Medical Association will be held in Chicago, June 11-15, 1956, with a full program of lectures, television, motion pictures, and scientific exhibits.

The lecture program for each Section is prepared by the Section Secretary, and prospective participants should communicate as soon as possible with the Secretary of the Section before which they hope to read a paper. Application blanks for space in the Scientific Exhibit are now available and may be obtained from the Section Representative to the Scientific Exhibit.

Additional information may be obtained from the Secretary, Council on Scientific Assembly, American Medical Association, 535 N. Dearborn St., Chicago 10.

**International Congress of Human Genetics.**—The First International Congress of Human Genetics will be held in Copenhagen, Denmark, Aug. 1-6, 1956. This congress is planned to cover all genetic aspects of normal and pathological characters in man. Any person interested in the subject of human genetics and especially of medical genetics is invited to take part in the congress.

The provisional program and further information will be sent on request to the Secretariate of the First International Congress of Human Genetics, The University Institute for Human Genetics, 14, Tagensvej, Copenhagen, N., Denmark.

**Regular Corps Examinations for Medical Officers, U. S. P. H. S.**—A competitive examination for appointment of Medical Officers to the Regular Corps of the United States Public Health Service will be held in various places throughout the country on March 20, 21, and 22, 1956.

Appointments provide opportunities for career service in clinical medicine, research, and public health. They will be made in the ranks of Assistant and Senior Assistant, equivalent to Navy ranks of Lieutenant (j. g.) and Lieutenant.

Entrance pay for an Assistant Surgeon with dependents is $6017 per year; for Senior Assistant Surgeon with dependents, $6918. Qualified officers are promoted at regular intervals.

Benefits other than promotions include periodic pay increases, 30 days annual leave, sick leave, medical care, disability retirement pay, regular retirement pay which is three-fourths of annual basic pay at time of retirement, and other privileges.

Active duty as a Public Health Service officer fulfills the obligations of Selective Service.

Requirements for both ranks are U. S. citizenship, age of at least 21 years, and graduation from a recognized school of medicine. For the rank of Assistant Surgeon, at least seven years

of collegiate and professional training and appropriate experience are needed. For Senior Assistant Surgeon, an additional three years, for a total of at least ten years of collegiate and professional training and appropriate experience, are required.

Entrance examinations will include an oral interview, physical examination, and comprehensive objective examinations in the professional field.

Application forms may be obtained from the Chief, Division of Personnel, Public Health Service, Department of Health, Education, and Welfare, Washington 25, D. C. Completed application forms must be received in the Division of Personnel no later than Feb. 10, 1956.

## PERSONAL

**Cecil W. Morgan.**—Cecil W. Morgan, Ph.D., a consultant in rehabilitation for the Veterans Administration and the U. S. Army, has been named Director of Adult Vocational Services of United Cerebral Palsy, it was announced today by Jack Hausman, of Great Neck, N. Y., President of United Cerebral Palsy.

Mr. Morgan comes to United Cerebral Palsy after two years of service as Professor of Physical Education and Director of Courses in Rehabilitation at Springfield College, Springfield, Mass., and as a consultant of the Veterans Administration in Washington, D. C.

In his new capacity, Mr. Morgan will help to work out a national program of improving methods of rehabilitating adults with cerebral palsy and of providing vocational guidance for their benefit. In addition, he will assist United Cerebral Palsy affiliates in expanding and establishing local facilities for the vocational training and placement of the cerebral palsied.

Until his release from active duty as Lieutenant Colonel, Medical Service Corps, in 1953, Mr. Morgan was Director of Reconditioning Activities at the U. S. Army Reconditioning Center in Garmisch, Germany. From 1946 to 1950 he served as Consultant in Physical Reconditioning with the Office of the Surgeon General, U. S. Army, in Washington, D. C. During World War II he was acting Director of Physical Education, University of Pittsburgh. During 1944 Mr. Morgan was Recreation Coordinator with the Federal Security Administration, the forerunner of the Department of Health, Education, and Welfare.

# Books

EDITED BY WILLIAM B. BEAN, M.D.

**Antibiotics Annual 1954-1955.** Edited by Henry Welch, M.D., and Felix Marti-Ibanez, M.D. Price, not given. Pp. 1154, with many illustrations. Medical Encyclopedia, Inc., 30 E. 60th St., New York 22, 1954.

This is a compendium of the papers presented at the Second Annual Symposium on Antibiotics held in Washington, D. C., Oct. 25-29, 1954. All of the 172 presentations, most of them reports of original investigations, are included. As might be expected, the full gamut of therapy with all of the antibiotics now in common use is covered, and a number of new antibiotics not yet widely employed clinically are described. Two new substances found in nature are reported to have anticarcinogenic properties. Also, the role of antibiotics in human nutrition is explored. Again emphasis is placed upon the adverse reactions of antibiotics, most serious of which seems to be severe allergic responses and deleterious dislocations of the bacterial flora in the body so that organisms insensitive to the antibiotic being used gain the ascendency. For those wishing to keep abreast of new developments in the antibiotic field this book is probably the most complete coverage in a single volume that could be obtained. Most internists should find the symposium of extreme interest and utility. Investigators in the infectious disease field will find it indispensable.

PAUL S. RHOADS, M.D.

**Review of Medical Microbiology.** By Ernest Jawetz, M.D., and others. Price, $4.50. Pp. 360, with 92 illustrations. Lange Medical Publishers, P. O. Box 1215, Los Angeles, 1954.

This book is designed to be practical and useful as a reference book of clinical infections and the chemotherapy of them. A background of basic science material sufficient for good understanding is provided. Of necessity, details are omitted. However, this briefness does not detract from the usefulness of this book or the thoroughness of it. It is written by three men who are authorities and can be highly recommended as a real help to the clinician and student alike.

CARL BILLINGS, M.D.

**Symposium on Atherosclerosis.** Auspices of Division of Medical Science, National Academy of Sciences, Publication 338. Price, $2.00. Pp. 249. National Research Council, 2101 Constitution Ave., Washington 25, D. C., 1954.

This attractive, inexpensive volume is the record of a symposium initiated by the Air Force Directorate of Research and Development. The 23 papers are arranged in 5 parts; each part ends with a summary, and each paper with a skillful editing of the separate discussions.

The selection and arrangement of the papers cover major areas of fundamental study in atherosclerosis and reflect the admirable judgment of the chairman, Dr. Irvine H. Page, and of the distinguished participants. The book is a unique collection of fact and authoritative opinion in a burgeoning and vexed area of study. As such, it will be an invaluable reference to investigators and clinicians dealing with vascular disease; practitioners and students will find in it answers to most of their questions on the status of this field. It lacks an index. In all other respects it is a great and, what is more, a useful bargain.

A. C. CORCORAN, M.D.

**Year Book of Pathology & Clinical Pathology (1954-1955 Year Book Series).** Edited by William B. Wartman, B.S., M.D. Price, $6.00. Pp. 486, with 180 illustrations. Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1955.

The "Year Book of Clinical Pathology," along with the numerous siblings in the Year Book family, has established for itself a well-earned place of importance for the busy physician trying to keep up with "the literature." The quality of any such book is determined first by the quality of the articles published in the general field, next by the selection, then by the adequacy of the abstracts, and, finally, by the editor's introduction and commentary on specific articles. The inclusion of original essays introduces variation into a system which tends to become very rigid. The selection of the articles is excellent and gives a good panoramic view of the areas of general pathology. The abstracts, though somewhat uneven, generally are adequate. The personal flavor

of the editor is revealed in his introductory essay and lucid and thoughtful commentaries, which are scattered widely and wisely throughout the book as editorial asides. Sometimes, just as in recorded discussions of a paper at medical meetings, the comment is more valuable than the paper itself. This volume gives a wide panorama of current activity and interest in pathology and clinical pathology. It should be read especially by physicians who are not pathologists and who have not kept up with current medical writings in the field.

WILLIAM B. BEAN, M.D.

**Kinderkrankheiten und Ernährung.** By Prof. Dr. Kurt Scheer. Price, 20 DM. Pp. 141, with 20 illustrations. Dr. Dietrich Steinkopff Verlag, Holzhof-Allee 35, (16) Darmstadt, 1955.

This book, Vol. 19 in a series of smaller texts for postgraduate education, has the expressed purpose of serving the busy practitioner as a quick guide in the newer developments of the nutritional aspects of pediatrics. In its 141 pages the author deals with a great variety of subjects, which are not all immediately related to nutrition, e. g., congenital heart disease, fetal erythroblastosis, etc.

The presentation of the digestive physiology, vitamins, and vitamin deficiency diseases does not differ remarkably from similar chapters in standard pediatric texts. In such a relatively small volume, the multitude and variety of covered subjects results in a regretable lack of practical details. Considerable space is also devoted to matters of general interest and development of concepts, often exceeding the more practical aspects of pathology, diagnosis, and treatment. Our newer knowledge and understanding in the quantitative aspects of fluid balance and electrolyte disturbance are not covered.

The most comprehensively presented chapter is that on infantile diarrhea, which is conceived as the "central problem" of pediatrics. The ideas of its etiology and pathogenesis are traced in historical sequence. The earliest concepts of its infectious origin assumed that the normal colon inhabitants, Escherischia coli, under certain conditions becomes pathogenic and are the general cause of infantile diarrhea. These ideas were followed by the theory of an alimentary causation. The author's own studies and those of others reestablish the importance of the infectious nature of infantile diarrhea.

In summary, the book provides interesting and intellectually stimulating reading, although for the necessary details of clinical management and diagnosis of nutritional disturbances in infancy and childhood the reader should be better served with one of our standard texts.

**Christopher's Minor Surgery.** Edited by Alton Ochsner, M.D., F.A.C.S., William Henderson Professor of Surgery and Chairman of the Department of Surgery, Tulane University of Louisiana School of Medicine, and Michael E. DeBakey, M.D., F.A.C.S., Professor of Surgery and Chairman of the Department of Surgery, Baylor University College of Medicine. Seventh edition. Price, $9.00. Pp. 547, with 251 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1955.

This book is well organized and conveniently divided into sections so that one can consult it with ease. Part 1, on general considerations, should be especially valuable to every surgical resident. Chapter 5, The Surgical Resident, could well be used by every hospital with a residency teaching program as a goal for training its residents.

Although the authors admit that the title of the book is not the best, the general context has changed to make it even less appropriate than it was for the earlier editions. Some of the more useful minor surgical procedures have been dropped, and many of the rare clinical entities have been included, which makes the book more nearly like a textbook of general surgery.

The illustrations are well picked and thereby add clarity to the book. It reads very easily and would be an excellent reference book, especially for the surgical intern or first-year resident.

**Differential Diagnosis of Internal Diseases.** By Julius Bauer, M.D. Ed. 2, revised. Price, $15.00. Pp. 987, with 66 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1955.

This book of some thousand pages contains a vast amount of valuable and scholarly medical lore. Dr. Bauer has done a remarkable piece of work, and this second edition bears witness to its favorable reception. Dr. Bauer takes up first in various sections leading symptoms such as hemorrhages, backache, and disorders of general feelings, and then there follow chapters on leading signs pertaining first to general appearance and then to various systems of the body.

Each section is followed by an excellent bibliography, and there are numerous pertinent illustrations. Under the chapter on the general appearance, for example, are considered such things as stature, obesity and leanness, endocrine and pseudoendocrine features, jaundice, cyanosis, and edema, each one being gone into in detail with numerous implications. One is constantly stimulated in looking over the book by the variety and depth of information therein contained.

**The Tubercle Bacillus in the Pulmonary Lesion of Man: Histobacteriology and Tuberculosis.** By Georges Canetti, M.D. Price, $8.50. Pp. 226, with 48 illustrations. Springer Publishing Co., Inc., 44 East 23rd St., New York 10, 1955.

This book, originally in French, appears as a revised and enlarged edition translated under the sponsorship of a group from the Rockefeller Institute and the New York Hospital-Cornell Medical Center. Drs. René Dubos and Walsh McDermott in their foreword, from which we quote, give the keynote of the book:

"With the discovery of the tubercle bacillus and of tuberculin allergy, it appears as if the high road had been reached toward a complete understanding of the genesis and course of the various types of tuberculous lesions. Unfortunately, the paths followed by the histopathologists and bacteriologists began to part shortly after these discoveries were made; the tissue changes in infection became the preoccupation of the first group of investigators, the characteristics of the etiologic agent the province of the other groups. As a result, the student of tuberculous disease is usually confronted with painstakingly detailed studies of either the tissue or the parasite, but he has to rely principally on his own imagination to relate the isolated facts concerning the two aspects of the infectious process. It is precisely the integration of histologic and bacteriologic knowledge which constitutes the central theme of Doctor Canetti's book."

In brief, the activities of the bacillus are discussed along with the development of the lesion. On the whole, the book is pretty heavy going, definitely for the specialist, but important. There are an extensive bibliography, good illustrations, and fine printing.

**The Lung: Clinical Physiology and Pulmonary Function Tests.** By Julius H. Comroe Jr., Robert E. Forster II, Arthur B. Dubois, William A. Briscoe, and Elizabeth Carlsen. Price, $5.50. Pp. 219, with 57 illustrations. Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1955.

Since World War II there has rapidly developed a scheme of pulmonary-function tests which quantitatively analyze the patient's ability to breathe. This development is analagous to that of liver-function tests and kidney-function tests.

"The Lung: Clinical Physiology and Pulmonary Function Tests," by Dr. Julius Comroe and co-workers, at the University of Pennsylvania, presents the system of analysis of the patient with breathing difficulties which has been in use in 1955, gives the reason behind individual procedures, and shows clinical situations in which the tests are helpful. The recent uses of alveolar ventilation, ventilation-blood flow ratios, blood-gas diffusion, and compliance studies are handled, as well as older ideas about lung volumes and blood gas analysis. Physicians who have been appalled by the complicated mathematical presentation of pulmonary physiology in review papers will be pleased with the frequent use of understandable diagrams. Ten case studies illustrate application of the tests. An appendix includes the mathematical exposition of these physiologic concepts.

Defects in the book are defects in the subject as presently understood; five years from now sections on the relation between physiologic measurements and the underlying lung anatomy, on prediction of postsurgical functional results, and on evaluation of industrial disability can be more explicit.

The many diagrams and the text are clear. This is the best available monograph on the subject for the general medical reader and an excellent summary for those just entering this special field.

**Prolonged and Perplexing Fevers.** By Chester S. Keefer, M.D., and Samuel E. Leard, M.D. Price, $5.50. Pp. 248. Little, Brown & Company, 34 Beacon St., Boston 6, 1955.

The problem of perplexing fever assails the specialist in internal medicine and the physician in general practice. At one time or another it confronts every practising physician. This book by Keefer and Leard fills a long-felt need for a systematic treatise analyzing the problem of the patient with obscure fever. From their rich clinical experience and from a review of the now substantial literature they have culled many useful facts. The presentation of numerous case

reports gives a good demonstration of the many types of problem and their final solution. One gets the impression from such a study that all the cases are solved, which is far from true. Many patients recover without any solution. Others may die without having the diagnosis discovered during life or at autopsy. Specific infections still head the list, but the pattern of puzzling fever that one encounters in contemporary practice has shifted dramatically over the past 15 years. We now encounter more examples of tumors, blood dyscrasias, lymphoma, collagen diseases, and anomalies of the heat-regulation mechanism. Formerly tuberculosis, syphilis, malaria, and other infections played a major role. There are only a few examples of careless capitalization, such as is the case of sulfobromophthalein (Bromsulphalein), the strange use of icteric index as though the index were yellow like an autumn leaf, the statement that the heart rate rather than the rhythm was regular, the curious redundancy of "desiccated thyroid extract," but these are minor quibbles. This book will certainly establish for itself an essential place in every medical library and among the books of thoughtful physicians. It will be the indispensable reference book for the practitioner and a most useful book for the intern and resident.

**Behandlung rheumatologischer Erkrankungen durch Anästhesie.** By Dozent Dr. Med. Egon Fenz. Price, not given. Pp. 112, with 18 illustrations. Dr. Dietrich Steinkopff Verlag, Holzhof-Allee 35, (16) Darmstadt, 1955.

This monograph is the fourth edition of a thorough treatise on treatment of "rheumatological diseases" by means of anesthesia with procaine (Novocaine) infiltration.

It is divided into two parts. The first part gives (1) a short historical review of local anesthetic therapy and (2) a discussion of all known pathophysiological mechanisms effective in local procaine therapy, wherein the influence and the ideas of Speransky and Ricker are obvious.

The second part is based on the author's own experience with over 4000 well-recorded and followed-up cases and over 37,000 procaine infiltrations. Here, he discusses the technique, dosage, and number of treatments required for optimal results. The few schematic illustrations are helpful in explaining the text.

It should be noted that the term rheumatological diseases is used in a rather loose fashion, comprising such entities as rheumatoid arthritis, degenerative arthritis, tuberculosis of joints and vertebra, osteoporosis, gout, lipomatosis dolorosa, and others.

**Diseases of the Thyroid Gland.** By Samual L. Cargill, M.D., and Mark Falcon Lesses, M.D. Price, $12.00. Pp. 500, with 68 figures and 9 plates. Oxford University Press, 114 5th Ave., New York 11, 1955.

The authors of this text are, indeed, to be commended for a concise yet all-inclusive presentation of thyroid disease. They have masterfully organized the material so that the physiological, biochemical, and anatomical alterations of thyroid function are excellently correlated with the clinical findings described.

The figures and illustrations have been thoughtfully chosen and carefully prepared to convey to the reader the classical examples of the functional, pathological, or clinical abnormality being emphasized.

The authors have been tireless in their review of the literature and in the compilation of the text's bibliography, of which there are ten parts. This book would be a valuable asset to any physician's library.

**Electrocardiografia.** By Dr. Emilio Araya. Price, not given. Pp. 425. Lopez & Etchegoyen, S. R. L., Junin N. 863 (R.20), Buenos Aires, Argentina.

This book, written by Dr. Emilio Araya (Miembro titular de la Sociedad de Cardiologia) is specially for general practitioners. It covers much of the basic knowledge of electrocardiography, and each chapter is devoted to an important and basic problem. The author has reviewed the most valuable world bibliography, giving preference to American authors, primarily Wilson, Goldberger, and Grant.

It contains some important contributions of Argentine cardiology, such as Arrighi's well-known classic report. The section on arrythmias is specially clear and of particular interest for students. On the other hand, recently advanced research on ECG in mitral stenosis, including the indications for surgical treatment, ECG in congenital heart disease, and normal ECG in newborn babies and children, is not very extensively treated.

As a whole, this book should prove valuable for Spanish-speaking students and general practitioners and also for cardiologists as a bibliographic reference book.

# Scientific Exhibits

## Intramural Electrocardiography.

MYRON PRINZMETAL, ALFRED GOLDMAN, RASHID MASSUMI, LOIS SCHWARTZ, REXFORD KENNAMER, LOUIS RAKITA, and JEAN-LOUIS BORDUAS, Cedars of Lebanon Hospital, Los Angeles, and City of Hope Hospital, Duarte, Calif.

**The exhibit is concerned with the genesis of the electrocardiographic deflection in man and dog with relation to spread of excitation from endocardium to epicardium, as determined by intramural potentials. It demonstrates the manner in which various components of the electrocardiogram develop and points out the contribution made to the genesis of the electrocardiogram by various myocardial layers. These fundamental observations explain the clinical electrocardiogram especially in coronary artery disease in a more rational light than previously possible.**

### DEPOLARIZATION OF THE NORMAL VENTRICLE

The classic theory of normal ventricular depolarization is illustrated diagrammatically. Depolarization is supposed to proceed at a steady, constant rate from endocardium to epicardium. Under such circumstances, a pure QS wave would occur in leads from the ventricular cavity; an R wave would occur in epicardial leads from the ventricular surface; RS waves would occur in all intramural leads between the cavity and surface. The large question mark indicates that this theory was formulated before the development of intramural electrocardiography.

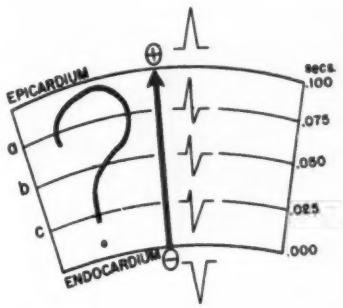



Fig. 1

According to classic theory, then, the R wave would be derived from and represent depolarization of the entire myocardial wall.

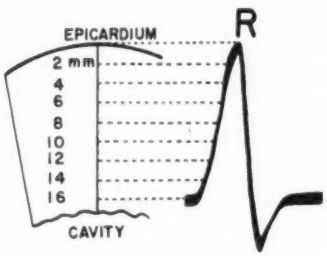



Fig. 2.

Figure 3 shows diagrammatically how the tiny intramural electrode may be inserted into the myocardium and record at any desired depth. The electrode is insulated except at its tip.

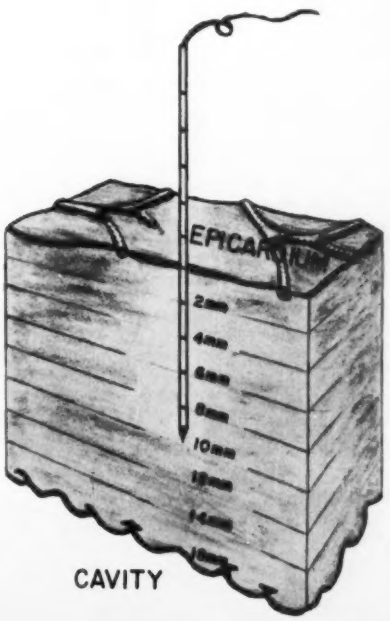



Fig. 3

Department of Medicine, School of Medicine, University of California, Los Angeles.

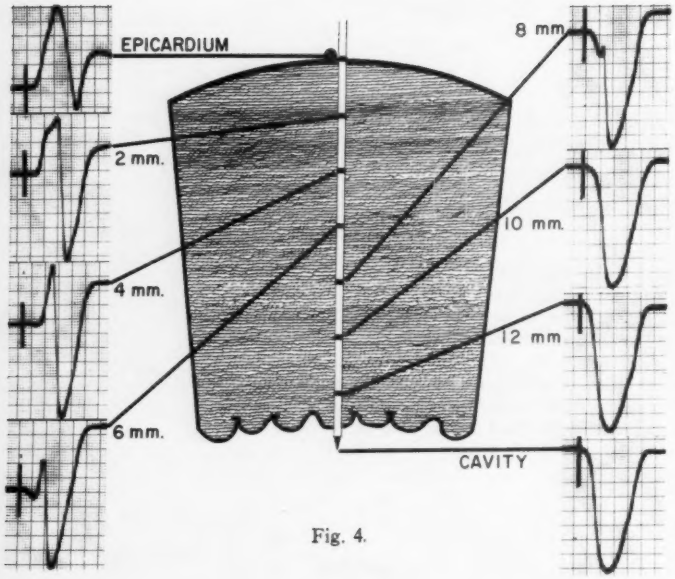



Fig. 4.

Exploration of the dog's left ventricle. Note that R waves are seen in complexes only from outer portion of wall. The inner layers record only QS waves.

Speed of impulse as measured by the onset of the intrinsic deflection at various levels in left ventricle of dog. Transmission is extremely rapid in inner layers. Rate of impulse transmission gradually decreases in outer layers.

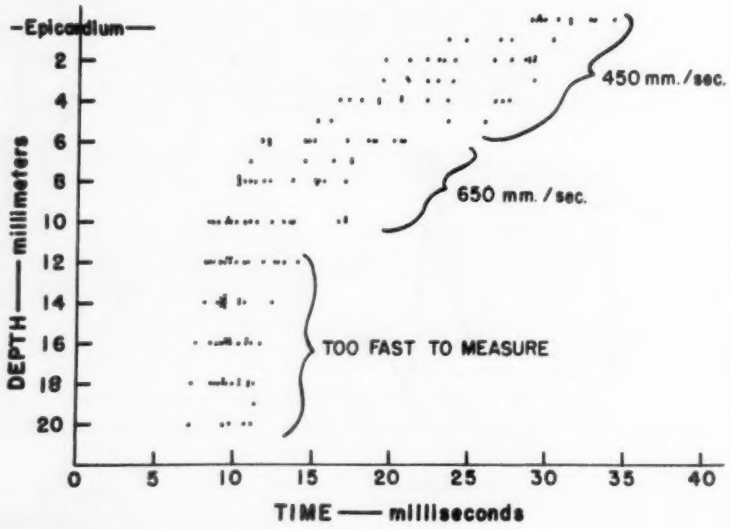



Fig. 5

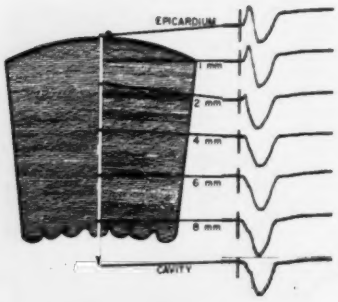



Fig. 6

Exploration of right ventricle of dog myocardium. As in left ventricle, QS wave similar to that of cavity persists until outer zone where R wave is recorded.

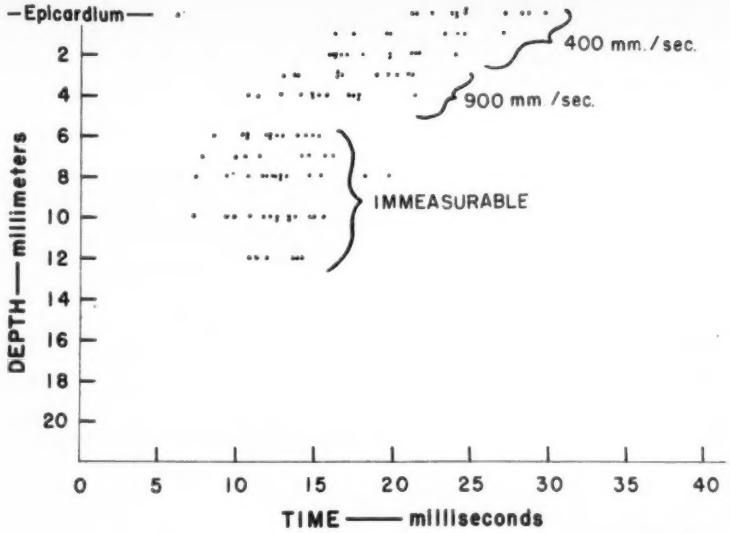



Fig. 7

Similar to left ventricle, speed of impulse transmission in dog's right ventricle is very rapid in inner layers and proceeds at a gradually decreasing rate in the outer layers.

Exploration of the normal human myocardium. As in the experimental animal, QS waves are recorded from the inner layers and the R waves appear only in the outer layers.

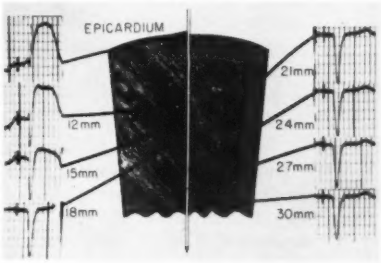



Fig. 8

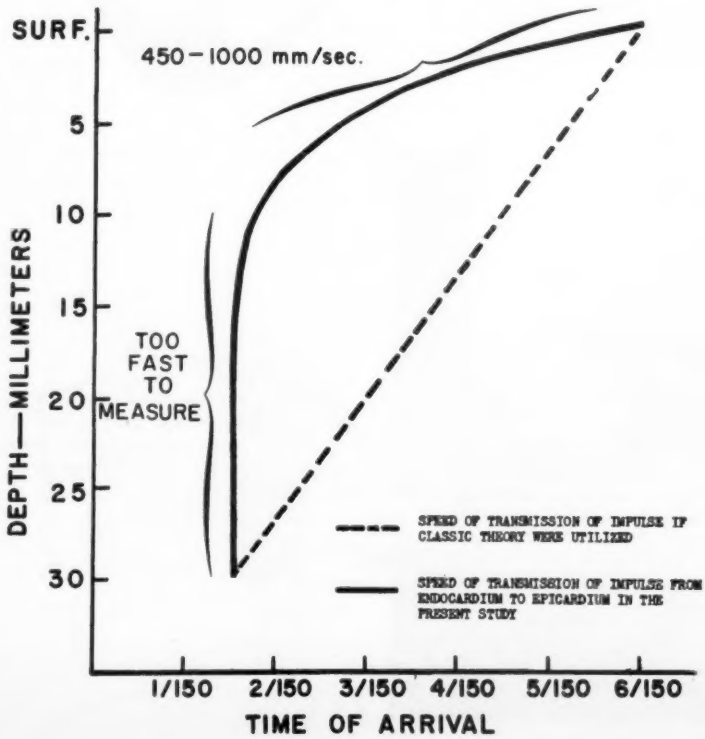



Fig. 9

If the impulse were transmitted at a constant rate from endocardium to epicardium as classic theory states, it would be represented by the broken line. Instead, actual measurements in the human ventricle show that the impulse is transmitted at a gradually decreasing rate in the outer layers and is extremely rapid in the inner layers.

The typical complexes recorded from various levels of the normal ventricle in both man and dog are represented diagrammatically. In accordance with classic theory, QS waves occur in cavity leads, R waves occur in epicardial leads, and RS waves occur in intramural leads from the outer layers. Intramural leads from the inner layers of the ventricle exhibit pure QS waves similar to the cavity complexes. They do not represent initial R waves. This indicates that depolarization of the inner ventricular layers is electrocardiographically silent; that is, for practical purposes it does not contribute to the clinical electrocardiogram.

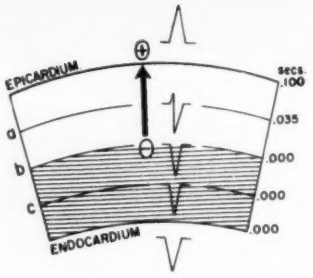



Fig. 10

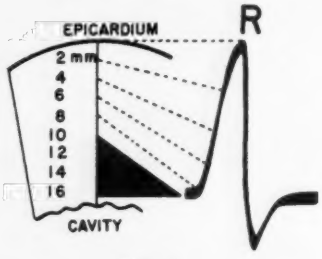



Fig. 11

Thus, the R wave is derived from the outer myocardial layers rather than the entire wall.

Theoretically, a reasonable explanation for the "silence" of the inner ventricular layers is that Purkinje fibers and branches may extend deeply into the subendocardium. Subendocardial depolarization might then occur so rapidly and in so many directions that no positive potential would be recorded.

Fig. 12

Fig. 13

## NORMAL T WAVE

Owing to extreme instability of T waves in open-chested animals, our experiments were carried out in an incubator providing constant temperature and humidity.

As diagrammed here, direct epicardial leads in the open-chested dog register inverted T waves in room atmosphere but upright T waves in the incubator, the latter T waves being similar to those in the precordial record when the chest is closed.

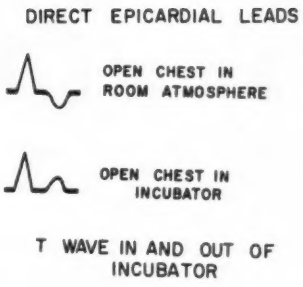



Fig. 14

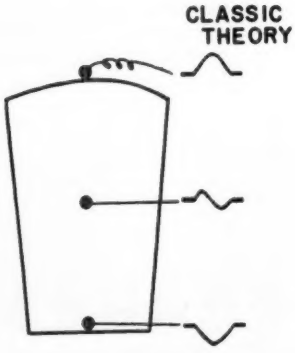



Fig. 15

Preliminary data suggest some discrepancies between classic theories and our findings. Theoretically, upright T waves should occur over the ventricle, biphasic T waves should occur in the midwall, and inverted T waves should be present in endocardial leads if repolarization proceeds from epicardium to endocardium.

The T waves recorded from the intramural layers of the human myocardium were identical in direction and amplitude in most cases.

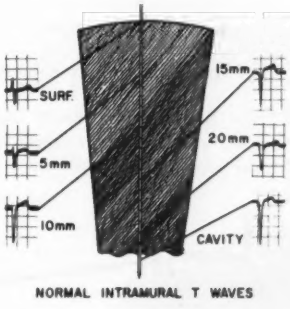



Fig. 16

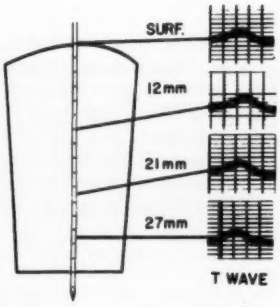



Fig. 17

Experimentally, the subepicardial and subendocardial T waves are often identical in direction and different in timing, the former usually being a few hundredths of a second later than the latter as illustrated here.

The T waves recorded in clinical electrocardiography appear to reflect directly the epicardial T waves. The body surface T waves, therefore, are probably governed by the repolarization of the superficial myocardial layers and are probably not influenced appreciably by repolarization of the subendocardium, just as the QRS of the clinical electrocardiogram is a reflection largely of the state of the epicardial layers.

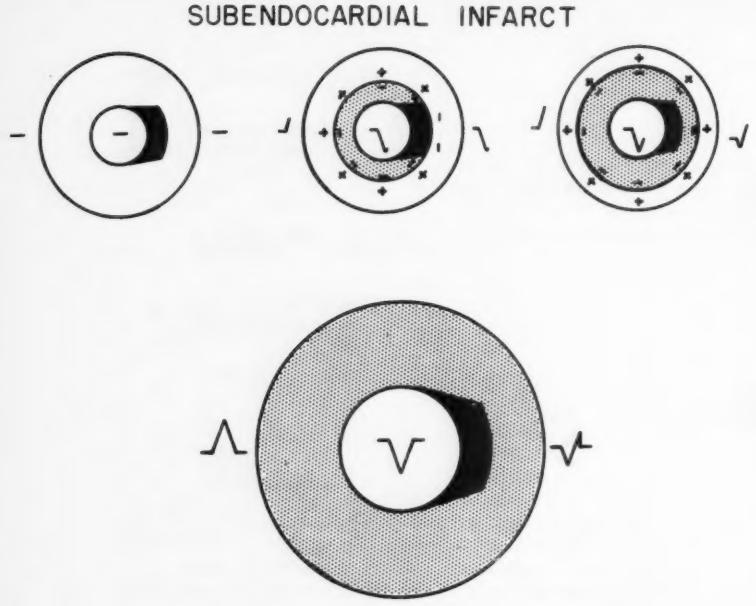



Fig. 18

**SUBENDOCARDIAL INFARCTION**

According to classic theory, subendocardial infarcts yield coronary QR waves. Transmission of the negative cavity potential through the subendocardial infarct presumably causes an initial Q wave. Depolarization of the intact outer layers over the subendocardial infarct then generates a positive potential, causing an R wave after the Q.

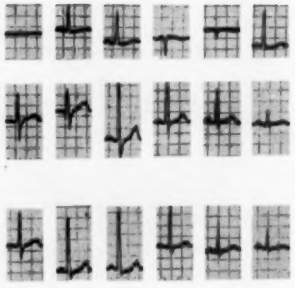


Fig. 19

The precordial electrocardiogram recorded over a ventricle with a subendocardial infarct is reproduced here. These tracings are typical of those from seven dogs with chronic pure subendocardial infarcts produced by coronary artery ligation. Note that the tracings are entirely normal.

The first row is standard limb leads 1, 2, 3, aVr, and aVf. Second row represents precordial leads, V1 through V6. The third row, tracings taken one interspace lower V1 through V6.

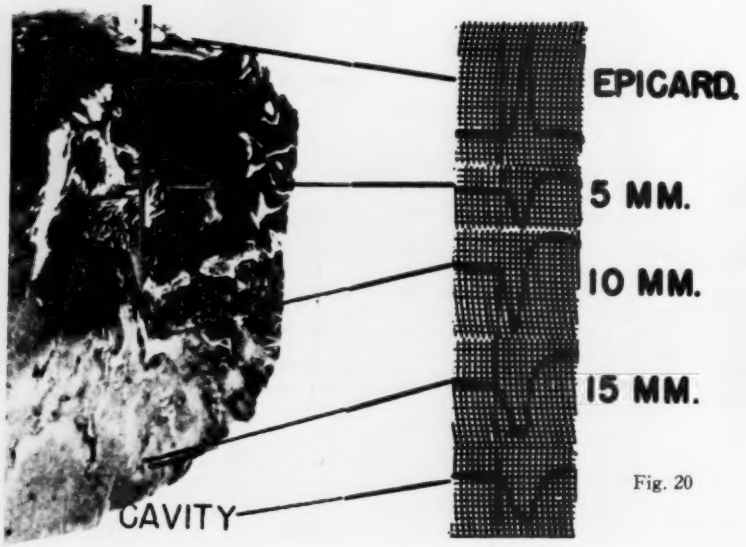
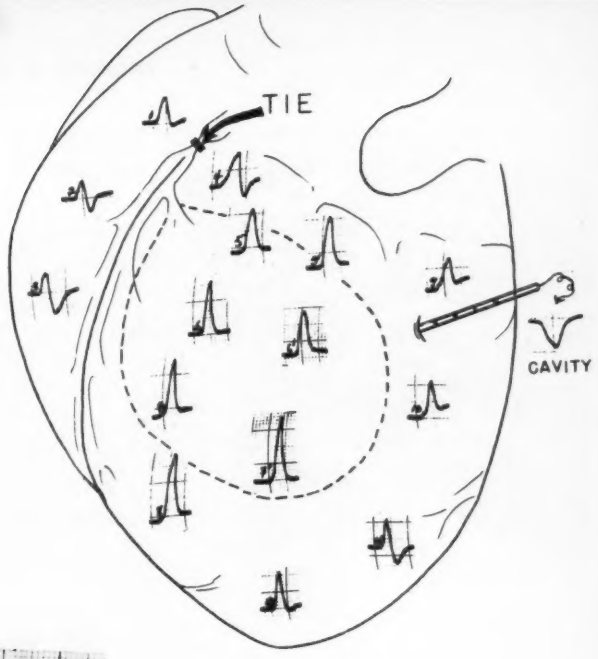


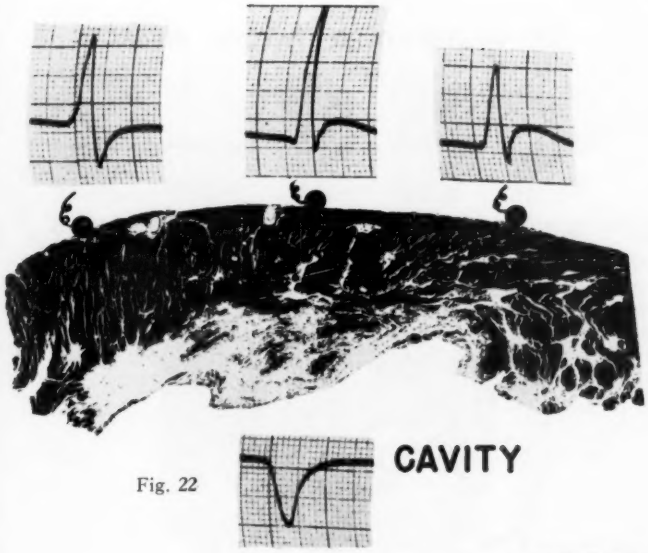

Fig. 20

Cross section of dog myocardium with experimentally produced pure subendocardial infarction. Epicardial lead registered over the infarct is normal. The fibrous region appears white (Mallory connective tissue stain.)




Diagrammatic representation of complexes from epicardial leads over entire surface of heart in a dog with pure subendocardial infarction, the extent of which is indicated by dotted line. All complexes are normal.

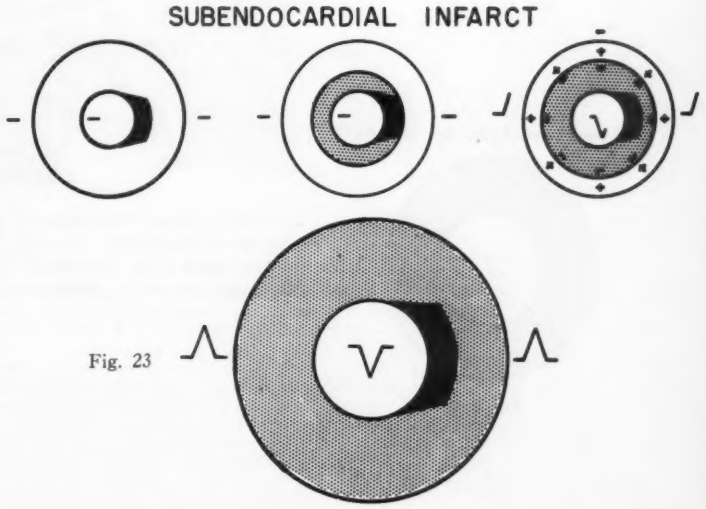
Fig. 21



Normal tracings from epicardial leads over pure subendocardial infarction in dog. Fibrotic tissue is white.




Fig. 22




Thus, pure subendocardial infarcts do not yield coronary QR waves. This is explained with reference to the observation that deeper layers of the normal ventricle are "electrocardiographically silent." Depolarization of normal subendocardial muscle does not contribute to the electrocardiogram. Pure subendocardial infarcts therefore cannot alter the normal depolarization complex. Clinically, patients with endocardial elastofibrosis may have a fatal outcome without ever registering an abnormal Q wave electrocardiographically.

Fig. 23

## CORONARY QR WAVES

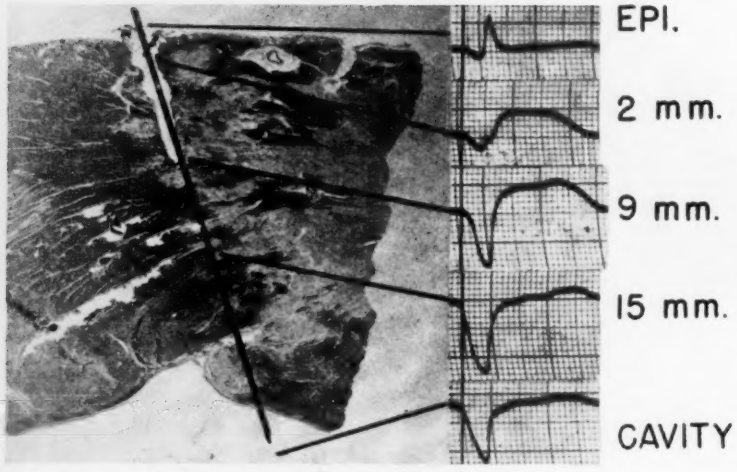



Fig. 24

Although coronary QR waves did not occur over pure subendocardial infarcts, they were nevertheless recorded from many animals after coronary artery ligation. In every instance, a mixture of live and diseased muscle was present in the outer ventricular layers. In Figure 24, patchy fibrosis is present in the light gray areas. The subendocardial muscle over which QR waves were recorded varied from completely normal to completely dead. Thus, coronary QR waves occur when the outer ventricular layers contain both live and diseased muscle. These deflections are independent of the status of the subendocardium.

Direct observations in human myocardial infarction support these experimental findings. In 5 patients with healed myocardial infarction and intractable angina pectoris undergoing surgery for improvement of coronary circulation, coronary QR complexes were recorded only from areas visibly involved by patchy infarction.

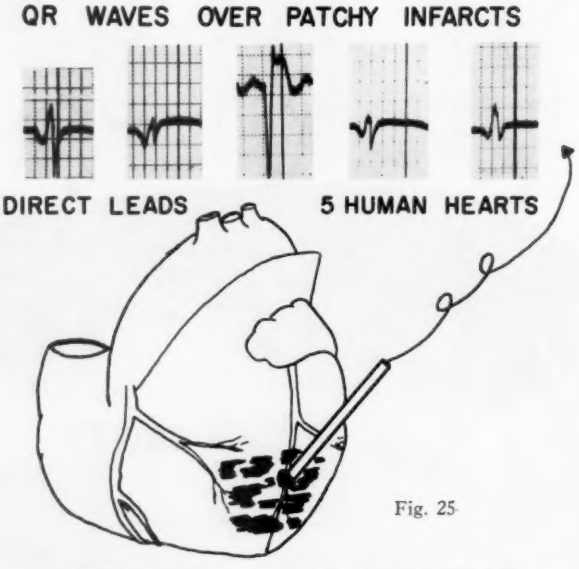



Fig. 25

CONFIRMATION of DOG EXPERIMENTS

THROUGH-and-THROUGH INFARCT

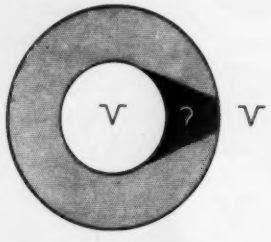


Fig. 26

### CORONARY QS WAVES

Classic theory concerning the genesis of coronary QS waves presumes that the negative cavity potential is transmitted unaltered through a "hole" of dead tissue to the epicardium. Coronary QS waves thus would signify that the underlying muscle, from endocardium to epicardium, is completely dead.

Cross section of dog myocardium in the gion of a through-and-through infarct. he wall is entirely devoid of live muscle. picardial, intramural, and cavity leads from is and other such lesions yielded identical S waves, confirming that the negative vity potential was transmitted unaltered rough the "hole" of dead tissue. The com- exes recorded over through-and-through farcts thus may be termed "cavity-type" ronary QS waves. Neither surface nor tramural ST segment elevation can be cited from myocardium with this kind of sion.

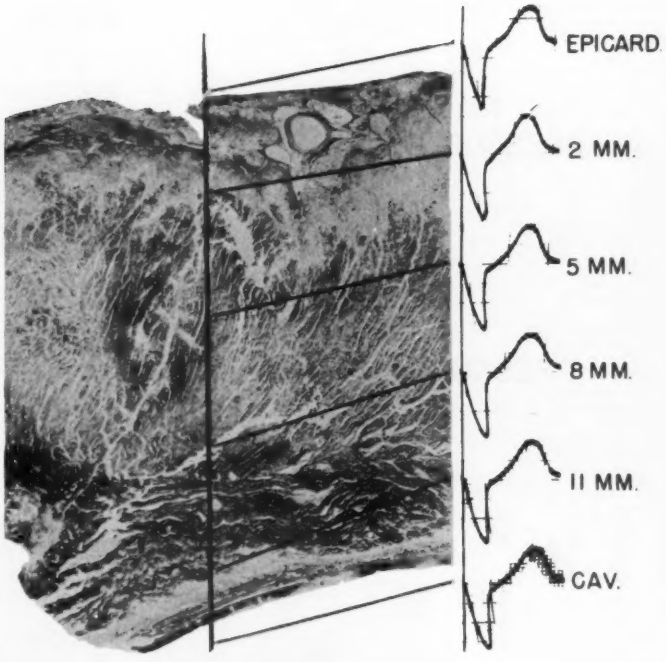



Fig. 27

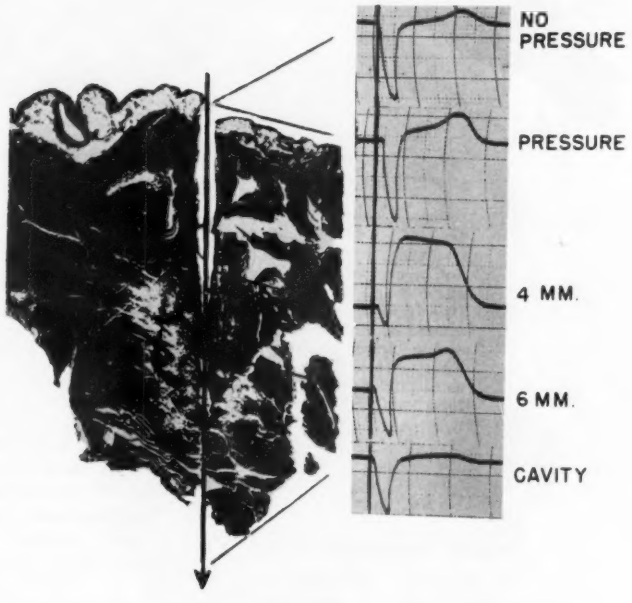
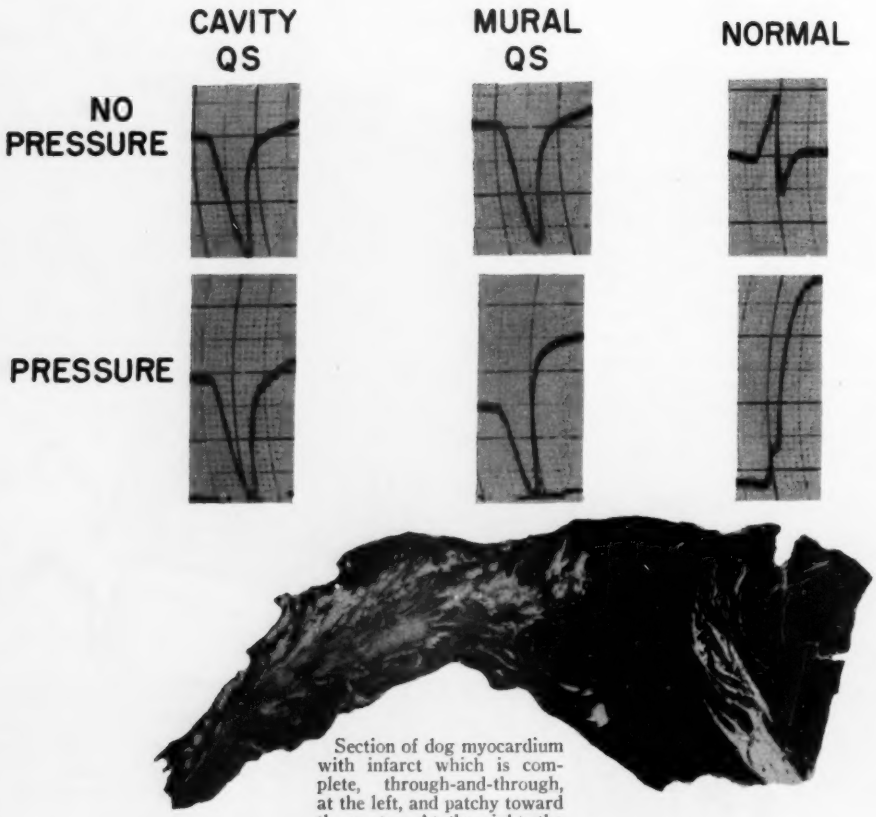



Fig. 28

A second type of infarct also yielded coronary QS waves. Here, ligation of the coronary artery produced a patchy infarct with significant amounts of live muscle scattered throughout the involved region. The darker-staining areas represent living tissue. As in through-and-through infarction, the negative potential presumably was transmitted from the cavity through the infarct. This negative potential, however, was modified by potentials resulting from depolarization of live muscle in the ventricular walls: thus, surface pressure caused an injury effect in the surface and intramural leads. The QS waves recorded over patchy infarcts may be called "mural-type" coronary QS waves. They occurred as frequently as cavity-type QS waves over chronic infarcts in experimental animals.




Section of dog myocardium with infarct which is complete, through-and-through, at the left, and patchy toward the center. At the right, the myocardium is normal. The effect of pressure upon the surface leads at these three points is shown. The cavity-type QS wave remains unchanged; injury effect cannot be elicited over through-and-through infarcts. Potentials from the remaining live muscle in the patchy portion of the infarct resulted in an injury effect due to pressure, as did those from the normal myocardium.

Fig. 29

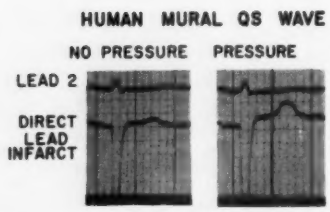
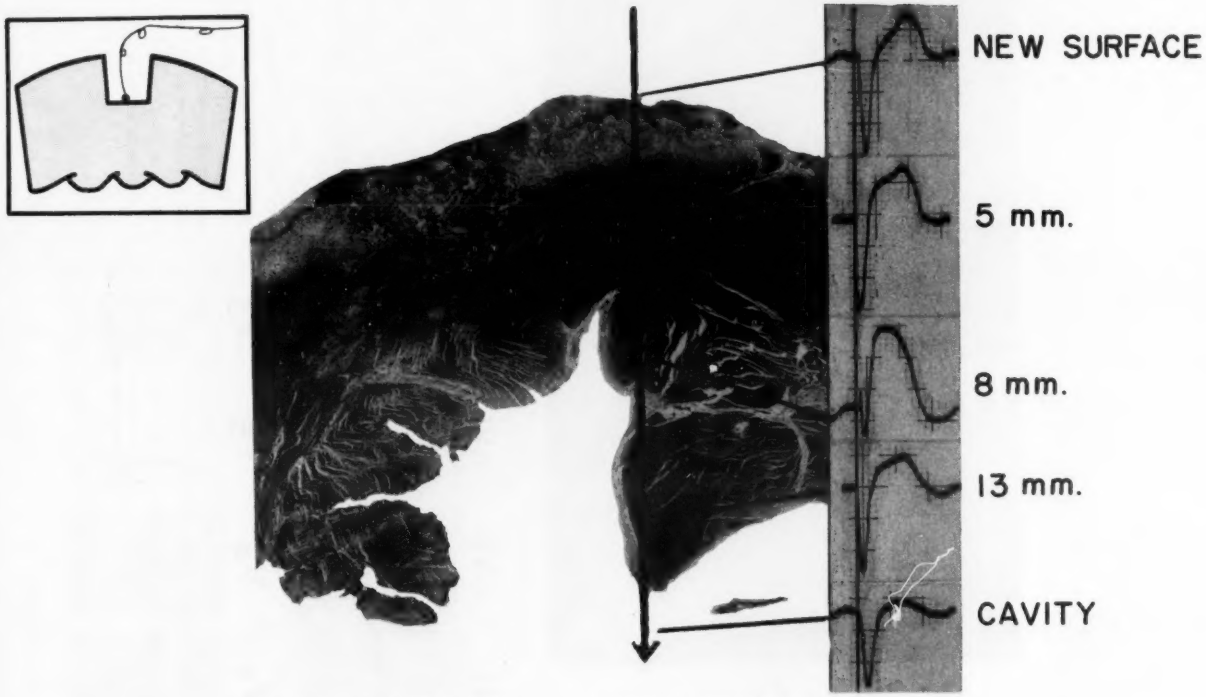



Fig. 30

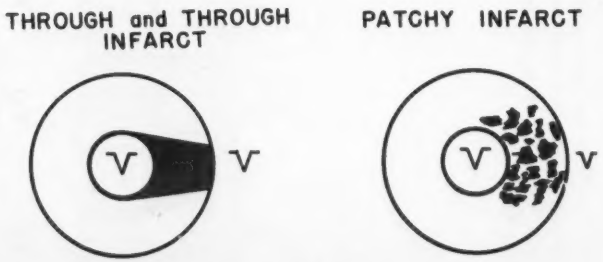
Human mural QS waves over the surface of an infarct obtained by direct leads during thoracotomy for cardiac surgery. Surface pressure caused injury effect indicating the presence of viable tissue in a region yielding QS waves. This is similar to the mural QS wave over patchy infarction in the dog shown in Figure 29.

Thus QS waves in man do not always indicate complete through-and-through myocardial death.




Section of dog myocardium in which a portion of the epicardial layer has been excised similar to the diagrammed inset. Note that, despite the fact that the underlying layers are intact and were able to function, a QS wave was obtained directly over the region devoid of epicardial layers.

Fig. 31




Contrary to classic theory, therefore, the cardiac muscle which yields coronary QS waves may not be completely dead and hence may retain its ability to function effectively.

Fig. 32

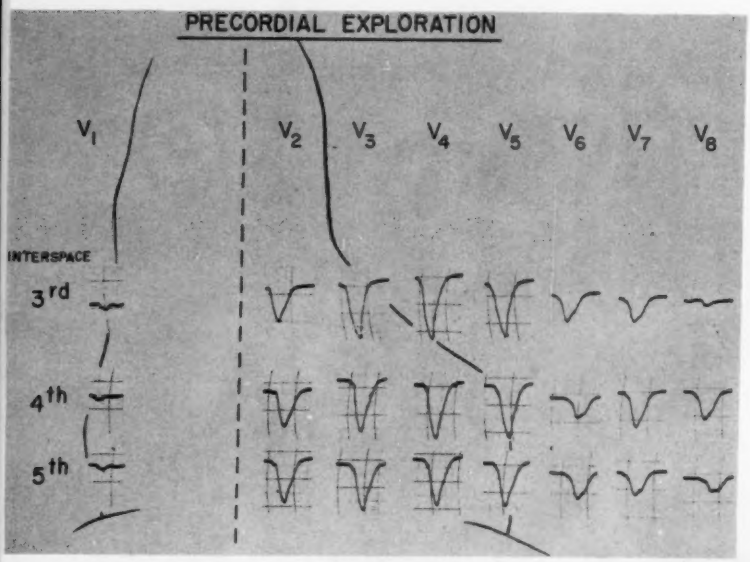



Fig. 33

Precordial electrocardiogram from a patient with infarction who recovered and became asymptomatic. These complexes were recorded at 125 mm/sec. (five times normal speed). If QS waves were always indicative of through-and-through myocardial necrosis, this patient could hardly have survived. These QS waves must be of the "mural-type," similar to those in the dog in Figure 28.

The patient whose electrocardiogram is shown in Figure 33 was found on high-speed cineradiographic examination to have a normal-sized heart which contracted efficiently.

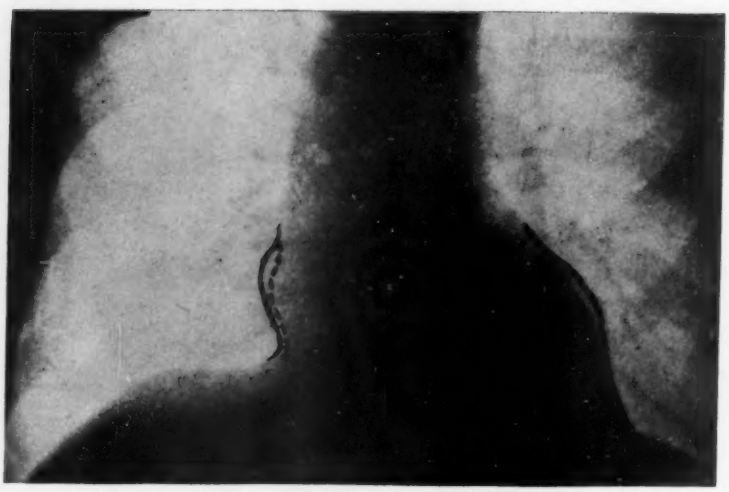


Fig. 34

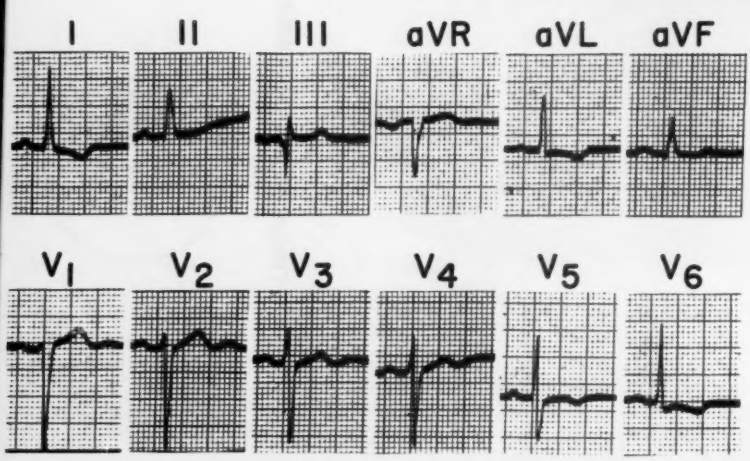



Fig. 35

On the other hand, this electrocardiogram is from a patient who had clinical evidence of five infarcts and subsequently died. The extensive damage to this patient's heart cannot be detected on the basis of his electrocardiographic changes.

Fig. 36

Thus, chronic subendocardial infarcts involving large portions of the ventricular myocardium may yield normal tracings if they are confined to the "electrocardiographically silent" zone. Contrariwise, less extensive damage to the outer ventricular layers may cause markedly abnormal complexes although the inner layers remain relatively intact.

Even the presence of pure QS waves cannot be regarded as unequivocal evidence that the underlying muscle is completely dead. These deflections may occur over portions of the heart containing enough live muscle to contract effectively as well as over through-and-through infarcts. This was clearly observed in high-speed motion pictures of infarcted ventricles recorded simultaneously with direct epicardial leads. The motion pictures revealed that through-and-through infarcts always ballooned outward during ventricular systole, indicating that they were noncontractile. In contrast, several patchy infarcts over which coronary QS waves were recorded showed active systolic contractions. By means of a Vanguard Analyser, it was possible to measure and illustrate diagrammatically the myocardial contraction associated with a given direct electrocardiographic complex.

The normal ventricular complex and the contraction curve are illustrated here.

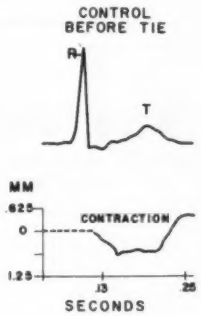



Fig. 37

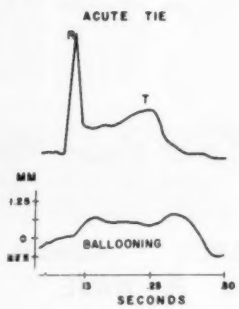



Fig. 38

After an acute coronary ligation in the dog heart illustrated in Figure 37, the involved region of the myocardium ballooned despite the persistence of an R wave in the direct lead from that region.

Contrariwise, in several dogs, patchy infarcts over which coronary QS waves were recorded showed active systolic contractions such as this. Since this myocardium could contract, these must be "mural-type" QS waves similar to those in man illustrated in Figure 35. Thus an R wave may be recorded from noncontractile myocardium, and myocardium which contracts effectively may register QS waves.

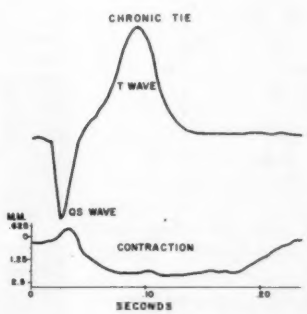



Fig. 39

From a practical viewpoint, these observations on myocardial infarction explain many discrepancies between classic electrocardiographic theory and actual electrocardiographic findings. They serve to emphasize that the physician cannot rely upon the electrocardiographic findings alone in the diagnosis and prognosis of myocardial disease.

## MYOCARDIAL INFARCTION IN THE PRESENCE OF LEFT BUNDLE BRANCH BLOCK

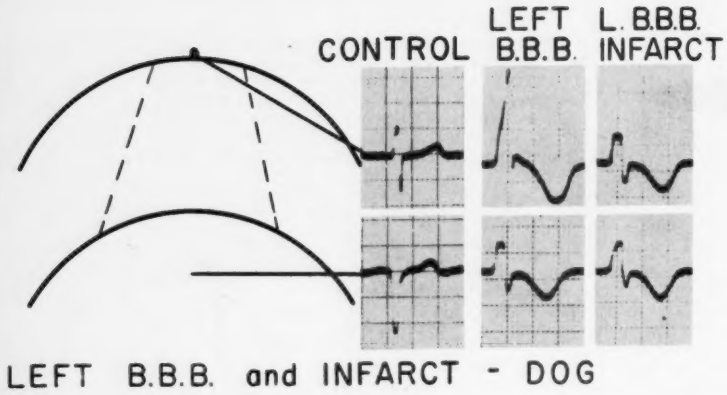



Fig. 40

It is classically stated that the initial positivity of the left ventricular cavity in left bundle branch block precludes the appearance of infarction Q waves when the left ventricle is infarcted, making the diagnosis of infarction by QRS changes in the presence of left bundle branch block impossible. As illustrated here, the cavity in dogs with left bundle branch block did show an initial positivity which was much smaller than the R recorded from the overlying surface. This was due to the positive potentials generated by the depolarization of the free left ventricular wall accounting for the greater amplitude in the left ventricular epicardial leads. When transmural infarction was superimposed on left bundle branch block, the left ventricular surface R wave was reduced in height, becoming approximately equal to the R wave of the left ventricular cavity as illustrated here. Therefore, in the presence of left bundle branch block, a decrease in R amplitude in leads from the left ventricular surface indicates superimposed infarction of the left ventricle.

In a few patients with left bundle branch block who developed myocardial infarction, the R waves recorded from the left lateral chest decreased in amplitude. The electrocardiogram from one of these is shown opposite. Compare leads $V_5$ and $V_6$ before and after infarct. This finding appears to have the same diagnostic significance in human beings as it has experimentally.

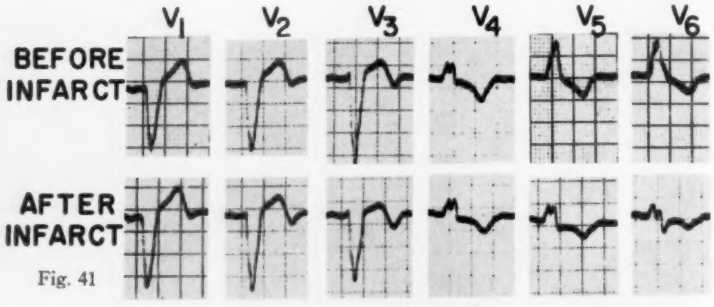



Fig. 41

LEFT B.B.B. and INFARCT - HUMAN
NOTE DECREASE IN R WAVE

## ABDOMINAL AND LUMBAR LEADS IN DIAPHRAGMATIC INFARCTION

There is considerable uncertainty in the interpretation of the Q waves in Leads III and $aV_F$. Deep wide Q waves in these leads may be caused by electrical positional changes of the heart while the absence of abnormal Q waves in these leads does not rule out infarction. Multiple-lead full body explorations in patients with well-documented myocardial infarctions showed that, in some cases, some of the abdominal and lumbar leads contained diagnostic Q waves while Leads III and $aV_F$ showed normal complexes. Abdominal and lumbar leads may be superior to $aV_F$ and III for the diagnosis of diaphragmatic infarctions because (1) abdominal and lumbar leads are closer to the diaphragmatic surface than are III and $aV_F$, and (2) abdominal and lumbar leads can be taken from many positions. The variety of angles that these leads subtend with the diaphragmatic surface of the heart offer a greater chance of uncovering QRS changes of the diaphragmatic infarcts, particularly when the infarcts are small. The left flank or lumbar lead seems most promising.

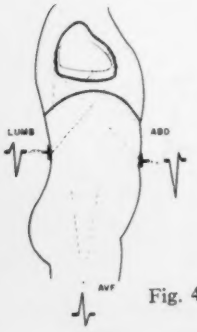


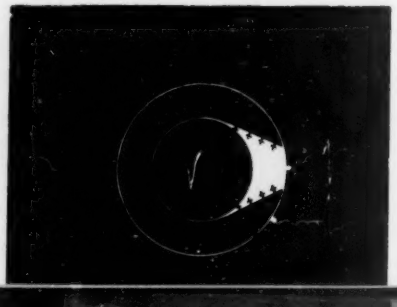

Fig. 42

ABDOMINAL and LUMBAR LEADS FOR DIAPHRAGMATIC INFARCTS

Fig. 43.

## ST SEGMENT DEVIATION

The ST segment changes observed in patients with coronary artery disease are generally attributed to a dipole at a boundary between injured and uninjured muscle. ST segment elevation presumably occurs if the electrode faces the injured side of the boundary. ST segment depression is supposed to occur when the electrode faces the uninjured side of the boundary. Leads recorded over transmural injuries and over the opposite wall thus should present elevated ST segments, while leads recorded adjacent to a transmural injury and over subendocardial injuries should present depressed ST segments.



The actual distribution of injury potentials immediately after coronary artery ligation is shown here. As indicated by the plus signs, elevated ST segments occur throughout the injured region. The amount of elevation diminishes from epicardium to endocardium and from the center to the border of injury. This gradient of positivity exists even though the inner ventricular layers suffer at least as much anoxia as the outer layers. No such gradient would occur according to the theory illustrated in Figure 43. Also, contrary to classic theory, ST segment depression usually does not occur adjacent to the injury, and ST segment elevation does not occur over the opposite wall. Instead, leads recorded outside the boundary of injury are usually entirely normal.

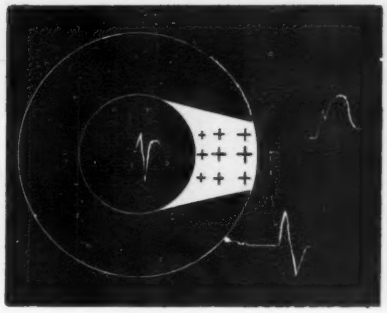


Fig. 44

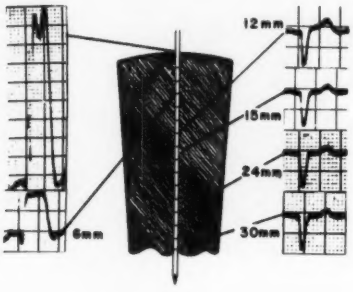



Fig. 45

Surface and intramural tracings from a human heart during cardiac surgery. The application of slight pressure with the surface electrode caused marked injury potentials at the surface and in the outer myocardial layers, but slight or none in the inner layers.

In the dog heart, just as in the human heart, injury potentials in response to surface pressure were marked in the epicardial layers and minimal to absent in the inner layers.

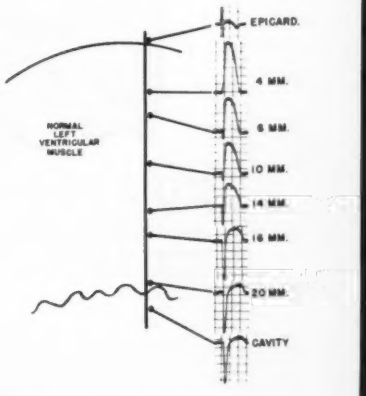
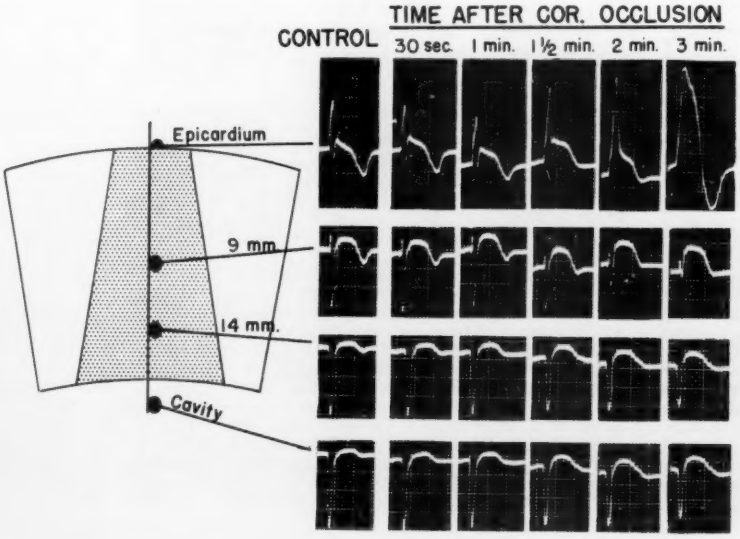
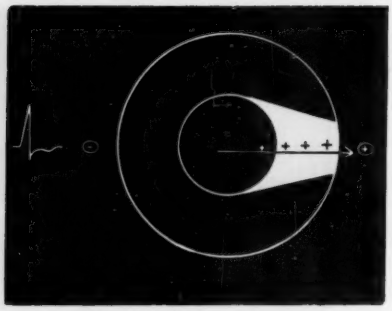



Fig. 46




As illustrated diagrammatically in Figure 42, the injury potentials which appeared in the involved region immediately subsequent to coronary artery ligation in the dog were most marked at the surface and in the epicardial layers and the amount of elevation diminished from epicardium to endocardium, despite the fact that the inner layers were probably more extensively involved.

Fig. 47



Direct leads taken over the wall opposite an infarcted region usually showed slight to moderate ST depression. The ST depression recorded opposite an injury is of the reciprocal type which can be related to a vector. Since positivity within the ventricle diminishes as the distance from the injured epicardium increases, the wall opposite the injury is relatively negative and therefore yields depressed ST segments.

Fig. 48

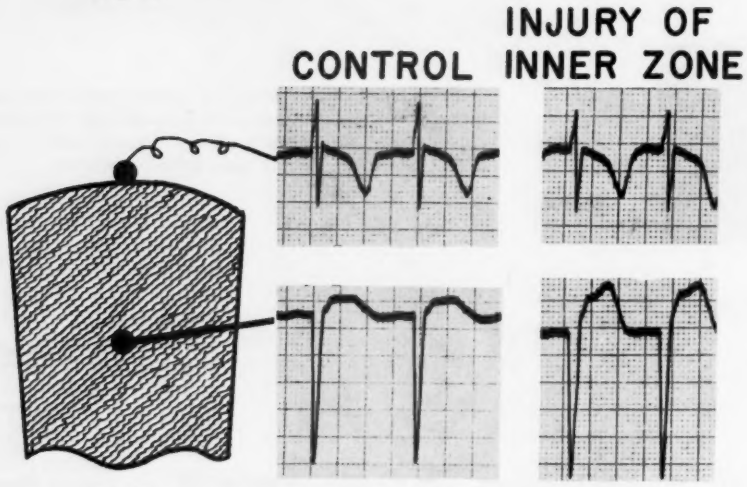



Fig. 49

Normal muscle over subendocardial injuries failed to yield depressed ST segments. The presence of subendocardial injury was established by ST segment elevation in subendocardial leads. Instead of reciprocal depression, isoelectric ST segments occurred in surface leads over the subendocardial injury.

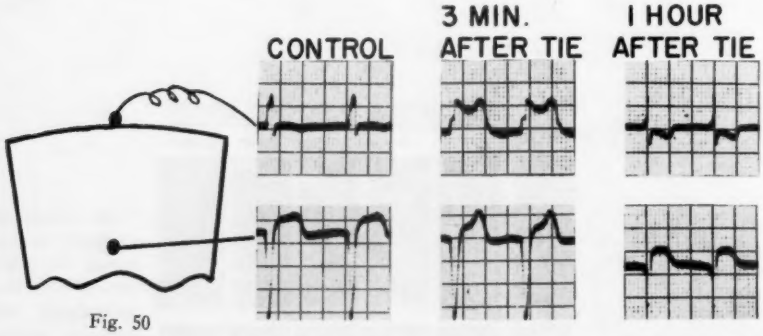



Fig. 50

In contrast to reciprocal ST segment depression, a type of ST segment depression was observed which is tentatively termed "primary epicardial ST depression." This type of depression was obtained in experimental animals under a variety of circumstances and also in human beings. It was detected only from the epicardial surface and appears to be due to certain functional or biochemical changes taking place in the outer layers of the myocardium. This ST depression may be related frequently to myocardial ischemia but may also appear in response to physical changes, such as the application of moderate heat to the myocardial surface. See surface complex under 40 C in Figure 56. It is not associated with ST elevation in other areas of the heart and is not related to the state of ST segments in the underlying subendocardial myocardium. Note that it appeared opposite after the injury effect had subsided and that leads from the underlying subendocardium did not change correspondingly.

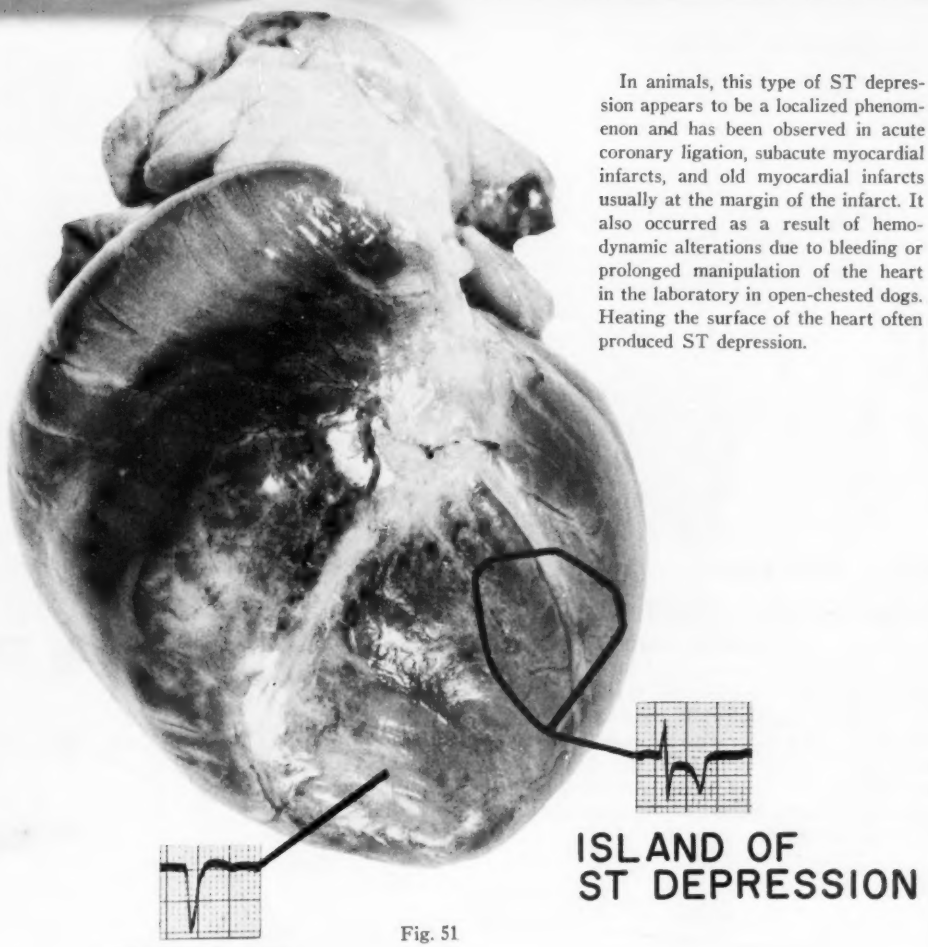
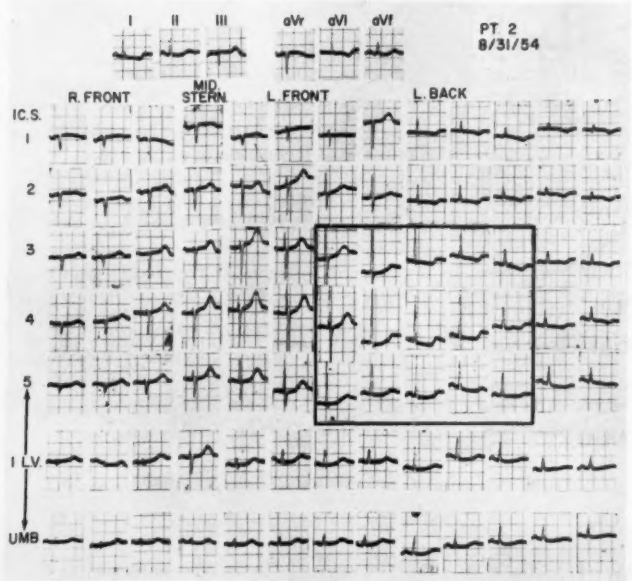



Fig. 51

In animals, this type of ST depression appears to be a localized phenomenon and has been observed in acute coronary ligation, subacute myocardial infarcts, and old myocardial infarcts usually at the margin of the infarct. It also occurred as a result of hemodynamic alterations due to bleeding or prolonged manipulation of the heart in the laboratory in open-chested dogs. Heating the surface of the heart often produced ST depression.




In patients, this type of ST segment depression has been observed in association with myocardial infarction a day or two before the appearance of ST segment elevation with typical QRS changes; in subacute infarcts after regression of the ST segment elevation, and in old infarcts long after the disappearance of the acute ST segment elevation. The isolated area of ST segment depression outlined here in a case which was explored thoroughly appeared in association with coronary-type chest pain. Two days later the ST depression was replaced by QRS changes indicative of acute infarction.

Fig. 52

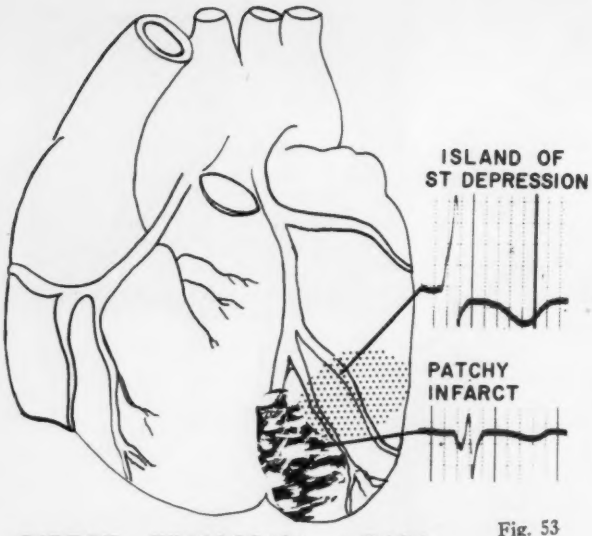



Fig. 53

DIRECT EPICARDIAL LEADS
HUMAN HEART DURING SURGERY

In three patients with old infarcts in whom the electrocardiogram showed ST segment depression, direct epicardial leads obtained during thoracotomy for surgical improvement of the coronary circulation recorded islands of epicardial ST depression on the anterior aspect of the apex as diagrammed. The areas of epicardial ST depression were found to lie directly under the areas of ST depression on the chest and were believed to be related. This type of ST segment depression, which usually occurs in islands adjacent to infarcts, appears to be a common finding in association with clinical myocardial infarction in all stages. Approximately one-third of all patients with myocardial infarction exhibit this type of ST depression either before, during, or after infarction. One may conclude that both experimental and clinical ST segment deviation appear to be a function of injury potentials generated in the outer myocardial layers and are not influenced appreciably by subendocardial changes.

### T WAVE CHANGES

It was observed that the electrical forces responsible for the T wave in normal man appear to be generated in the outer myocardial layers (Figs. 15-17). The human intramural T waves were usually identical in direction and amplitude, and a reciprocal relationship between the subepicardial and subendocardial T waves was only occasionally observed. T waves from two human cases subjected to cardiac surgery are shown here. In *A* and *B*, the T waves are similarly upright in the cavity and midmurally but differ in amplitude at the surface.

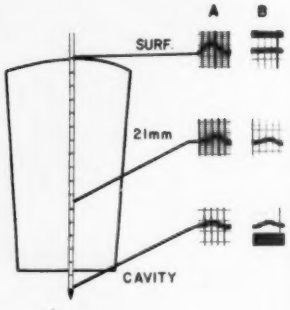
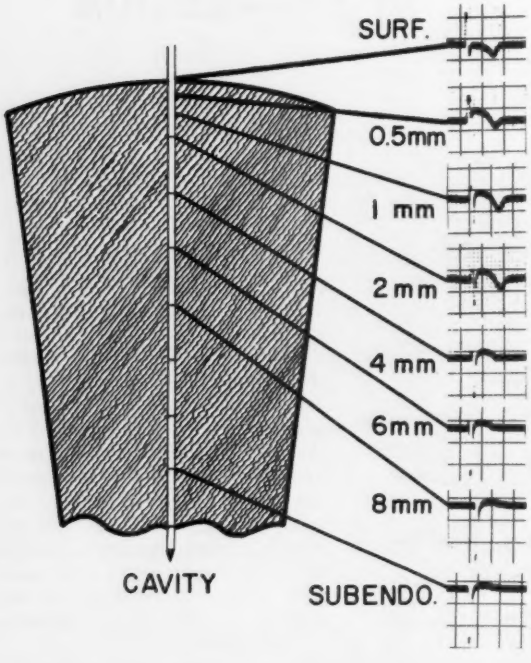



Fig. 54




INTRAMURAL T WAVES
At the Margin of an Old Infarct

Fig. 55

In a region presumed to be ischemic throughout at the margin of an old infarct in a dog, the intramural T waves apparently had no reciprocal relationship, just as in most of the human hearts. Here, ischemic-type T wave inversion in the outer layers is not reflected by similar or reciprocal T wave changes in the endocardial layers.

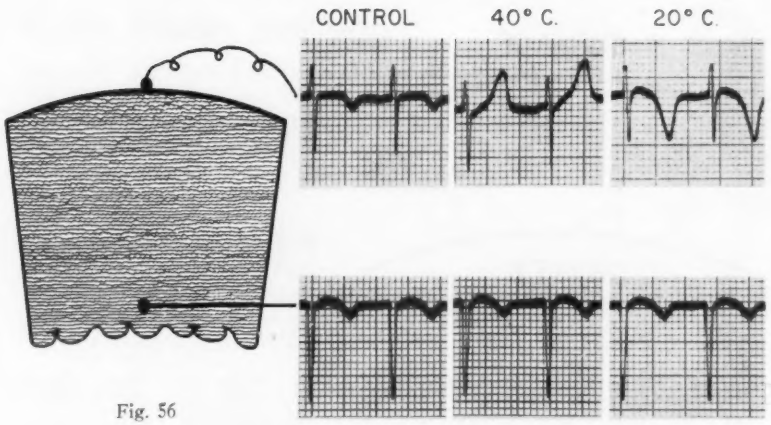



Fig. 56

The epicardial and subendocardial T waves showed remarkable independence both in direction and time of occurrence. Here alterations in duration and direction of the epicardial T waves in the dog's heart due to application of minute amounts of heat or cold to the surface had little or no influence on the subendocardial T wave.

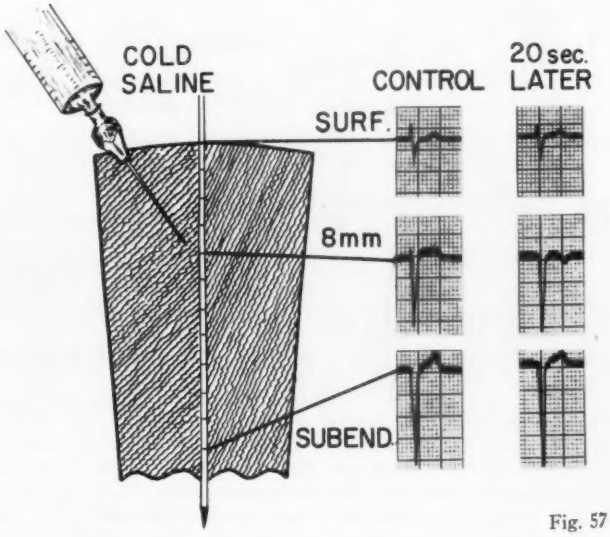
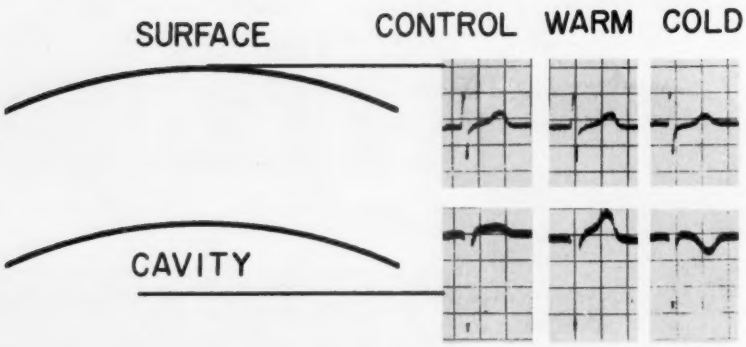



Fig. 57

## MIDMURAL INJECTION - COLD SALINE

Similarly, injection of a minute quantity of cold saline midmurally inverted the T wave recorded at that region but did not affect the surface or subendocardial T waves.




INJECTION OF WARM AND COLD SALINE IN LEFT VENTRICULAR CAVITY

Fig. 58

And, finally, injections of warm and cold saline into the left ventricular cavity of the dog's heart resulted in characteristic changes in the subendocardial T wave often without affecting the epicardial T wave.

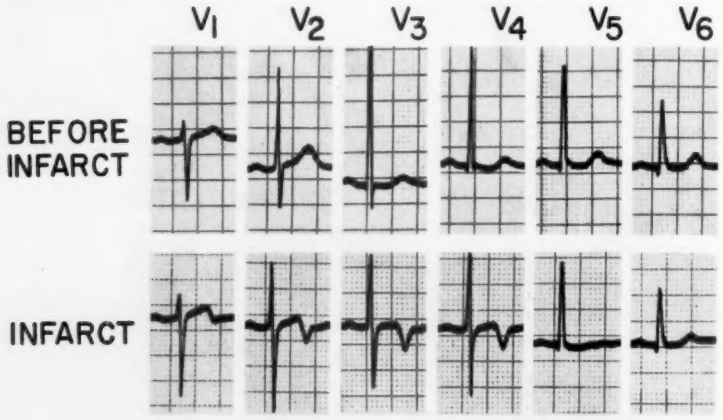


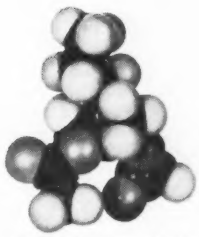

Fig. 59

DECREASE IN R AMPLITUDE ASSOCIATED WITH T INVERSION

Clinically, the presence of abnormally inverted T waves in association with diminished R wave amplitude appears to be more significant than T wave changes alone. Note the decreased R wave amplitude in this case after infarction. This decreased R amplitude accompanying infarction T wave inversion has also been observed experimentally. These changes may be useful in some cases in differentiating between T wave inversion associated with physiologic effects, such as postprandial T wave inversion, etc., and infarction T wave changes.

**This is one of the many exhibits shown at the June, 1955, meeting of the American Medical Association and which have been collected for pictorial presentation in the "A.M.A. Scientific Exhibits 1955." This book is published by Grune & Stratton, Inc., N. Y. These exhibits are published in their entirety and are not abstracts.**

*Two articles in the April 30th issue of The Journal of the AMA [1,2] report on . . .*



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1. Selling, L. S.: J.A.M.A. **157:** 1594, 1955.

2. Borrus, J. C.: J.A.M.A. **157:** 1596, 1955.

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The ataractic, tranquilizing action of 'Thorazine' can reduce the anguish and suffering associated with bursitis. 'Thorazine' acts not by eliminating the pain, but by altering the patient's reaction—enabling her to view her pain with a "serene detachment" . . . Howell and his associates[1] reported: "Several of [our patients] expressed the feeling that ['Thorazine'] put a curtain between them and their pain, so that whilst they were aware that the pain existed, they were not upset by it."

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*Smith, Kline & French Laboratories, Philadelphia*

1. Howell, T.H.; Harth, J.A.P. and Dietrich, M.: Practitioner *173*:172.
*T.M. Reg. U.S. Pat. Off. for chlorpromazine, S.K.F.